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TSETSE

The future for biological methods in integrated control

Editor: Marshall Laird

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Editor:

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Foreword

The appearance of this publication on the potential for biological control of the tsetse fly is very timely because of the increasing emphasis on research in the tropical diseases field, an area that has been sorely neglected in the past 15–20 years. It arose from a Scientific Advisory Group meeting on the role of pathogens, parasites, and predators in tsetse control, held in March 1974 and supported by the International Development Research Centre. Scientists from many disciplines have contributed to this work, which is a comprehensive review of the biology of the tsetse fly.

This publication should be extremely useful to those involved in applied entomology, and especially those working in vector control.

J. Gill, M.D.,
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Tsetse: The future for biological methods in integrated control

Preface

Classically, biological control (or biocontrol) has been largely associated with the deliberate use of predators, parasites, and pathogens as agents in the control of insect pests and disease vectors. To date, this branch of applied entomology has had far greater impact in the economic than in the medical aspects of the subject. Public health entomology boasts some successes, though mostly on a rather limited scale, and mostly relating to mosquitoes. Certainly, it has no achievements to compare with, for example, the spectacular saving of California's citrus industry from scale-insects through the importation of Australasian coccinellid beetles almost a century ago; or the reclamation of huge areas of Australian and South African pasture-land from prickly-pear cactus by the importation of *Cactoblastis* moths from South America in the 1920s.

This is partly because mankind's dispersal of economically important plants about the world has often resulted in the accidental dispersal of pests and weeds, which in varying degree have proved susceptible to control by means of the introduction and establishment of their natural enemies (and latterly, diseases), from the original homeland. Many of our prime vector-borne disease problems pose the need to impose drastic control — occasionally pressed to the point of local eradication — upon insects (such as many mosquito transmitters of malaria, filariasis, and arboviruses, and all of continental Africa's tsetse transmitters of human sleeping sickness and domestic-animal nagana) securely established in areas to which they are indigenous. Moreover, some of the most important insect vectors of disease, all the mosquitoes and blackflies for example, are virtually free from attack by the tiny parasitic wasps and flies collectively known as parasitoids, which have proved so important as biocontrol agents in agricultural and forestry entomology.

Therefore, the biocontrol successes against these insects of health importance have tended to be associated with the manipulation of predator populations, notably larvivorous fish of the genera *Gambusia* and *Lebistes*. In fact, beyond the realm of chemical control, environmental manipulation by, for example, source reduction, has made a larger contribution insofar as medical entomology is concerned. However, the muscoid flies and their near relatives, tsetse flies included, *do* suffer a measure of natural population limitation by parasitoids. There have been many experimental studies of these relationships, and some modest practical successes; though very modest and short-lived at least for *Glossina*.

The post-World War II period saw the widespread introduction of synthetic chemical pesticides. These brought about a revolution in insect control. They opened prospects of our at last being able not only to maximize crop production in the face of an inexorably growing human population combined with a fairly static arable land resource, but also to break the chain of transmission of such vector-borne scourges as malaria, filariasis, onchocerciasis, and sleeping sickness. Two main obstacles to the attainment of these goals duly presented themselves: (1) the highly complex problems of the resistance

of insects to pesticides; and (2) the nonselectivity and environmental persistence of some of these compounds. (Lately, due to the oil crisis, another such obstacle has been raised: (3) escalating costs of pesticides.)

Meanwhile, the essentially new discipline of invertebrate pathology was emerging. Microbial control agents started to play an increasingly important role in biocontrol, alongside the "classical" agents already mentioned. Once again, though, the early achievements tended to be in economic rather than public health entomology. Stimulating epizootics among pests of monoculture crops continued to prove easier of attainment than among more diffusely scattered vector populations. Nevertheless, from the start, the potential of microbial control of medically important insects was recognized. Following promising small-scale field trials with disease-causing organisms against mosquitoes, the World Health Organization (WHO) issued an annotated list and bibliography of pathogens, parasites, and predators of medically important arthropods (Jenkins 1964) that provided an indication of the great diversity of candidate biocontrol agents awaiting intensive study.

Such study has been vigorously pursued since then. Public health entomologists, like their economic counterparts, are now generally agreed that the future lies with integrated control, within the broader parameters of pest management. This approach provides for the conjoint or sequential use of selectively applied chemical pesticides, biocontrol agents, and other new noninsecticidal procedures (e.g. based on synthetic insect growth regulators and genetic control), along with mechanical and other measures, in methodologies carefully designed not only for long-term effectiveness but also for health and environmental acceptability. Since 1972, there have been reports and compilations on the feasibility of incorporating biocontrol techniques in future integrated control programs against blackflies (IDRC 1972), mosquitoes (NAS 1973), and tsetse flies (IDRC 1974). There has also been some preliminary planning in this direction with respect to the control of the snail hosts of schistosomiasis.

The last-cited publication was the first outcome of a Scientific Advisory Group on "Tsetse Control: The Role of Pathogens, Parasites, and Predators," convened at The Memorial University of Newfoundland, St. John's, Canada, 25–29 March 1974, under the sponsorship of the International Development Research Centre (IDRC), Ottawa. This book is the second outcome of that meeting. It represents the first attempt to place between two covers the essential information on the status of existing knowledge of the natural enemies and diseases of one particular group of insect disease vectors, together with background information of ultimate relevance to the topic (i.e. pertinent facts about the taxonomy, distribution, bionomics, physiology, and control-history of that group), besides projections of the types of research and development likely to prove rewarding toward the early attainment of practical biocontrol procedures.

It is both encouraging to notice, and illustrative of the rapid maturing of the discipline of invertebrate pathology, that some notable events directly bearing upon this book's subject have taken place in the brief period since its contributors began their task less than 2 years ago. Data concerning virus-like particles from tsetse are rapidly accumulating, now that renewed interest is being taken in a fresh approach to integrated control, just as was the case with mosquitoes a decade ago. We can only hope that heightened awareness among those now handling large numbers of laboratory-reared *Glossina*, us-

ing sophisticated research technology, will be extended to large-scale surveys among "wild" tsetse and lead to the discovery of baculoviruses — nuclear polyhedrosis (NPV) and granulosis (GV) viruses—of interest as future microbial control agents. It would be an especially happy discovery at a time when these entomopathogenic entities have just been the subject of searching safety assessments. Out of this analysis has come the first-ever registration for a "viral insecticide" (U.S. Environmental Protection Agency Reg. No. 11273-17, with respect to ELCAR Sandoz Inc., Homestead, Florida), an isolate of the *Heliothis* nucleopolyhedrosis virus. The product was granted registration and label approval on 1 December 1975, for use against two crop pests, the cotton bollworm and tobacco budworm.

As the present work goes to press, an American product based on the mosquito worm *Romanomermis culicivorax* (Fairfax Biological Lab. Inc., Clinton Corners, New York) has been placed on the market in the USA as a contributor to the suppression of the larvae of many species of mosquitoes. Even more significantly, efforts are in progress to replace the present *in vivo* production of this mermithid (and others from blackflies) by *in vitro* technology. In this context, tsetse flies, while not yet known to be subject to baculovirus mortality, *are* parasitized by mermithids. Also, as recommended by the 1974 Scientific Advisory Group, an International Development Research Centre grant has been made (8 July 1975) to the Commonwealth Institute of Biological Control (CIBC) to explore the feasibility of using mutillid and bombyliid parasitoids of southern and southeastern African tsetse for *Glossina* abatement, notably in West Africa where *Mutilla* spp. are not yet known from these hosts.

Meanwhile, WHO has reaffirmed its intention to conduct field trials of bacterial and fungal microbial control agents, and selected larvivorous fish, against West African mosquito disease vectors. The Organization has also declared its interest in investigating the possibility of using biocontrol agents against tsetse flies. To this need only be added the fact that the 14th Meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), of the Organization for African Unity, convened at Dakar, Senegal, 15–17 April 1975, included among its recommendations not only "the creation of breeding units in Africa, capable of producing the principal species of *Glossina* on a large scale" (i.e. to facilitate basic research as well as genetic control) but also "that every effort should be made to intensify research on the possibility of using predators, parasites or pathogenic germs in biological control." It is hoped that our book may help toward this end.

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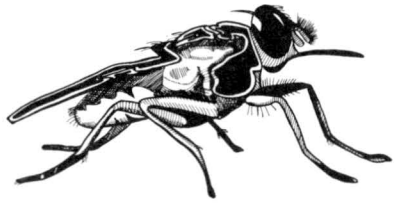


Systematics

All tsetse flies belong to the genus *Glossina* which, at present, is limited to Africa south of the Sahara-Somali deserts and north of the deserts of Southwest Africa, the Kalahari, and the seasonally cold tracts northeast of South Africa (see Distribution and Bionomics). One species, *G. tachinoides*, was recorded from Southern Arabia by Carter (1906) but its continued presence there has not been confirmed.

Tsetse flies are rather dull in appearance, varying in colour from a light yellowish-brown to a dark blackish-brown. Some species have transverse black bands on the dorsal surface of their abdomens. When alive and at rest two distinctive features of the genus are obvious. The wings are folded one over the other like the blades of a pair of scissors, with their tips projecting beyond the end of the abdomen; and the tubular mouth parts, with ensheathing palpi, project forward from under the head. The smallest species is 6–8 mm long; the largest, 10–14 mm. Details of the characteristics of the genus are given by Newstead et al. (1924). Two features clearly distinguish this genus from other Diptera: (1) the discal cell of the wing (the so-called "hatchet cell," characteristically shaped like a cleaver), which lies between longitudinal wing veins IV and V; and (2) the presence of secondary branches on the hairs of the antennal arista.

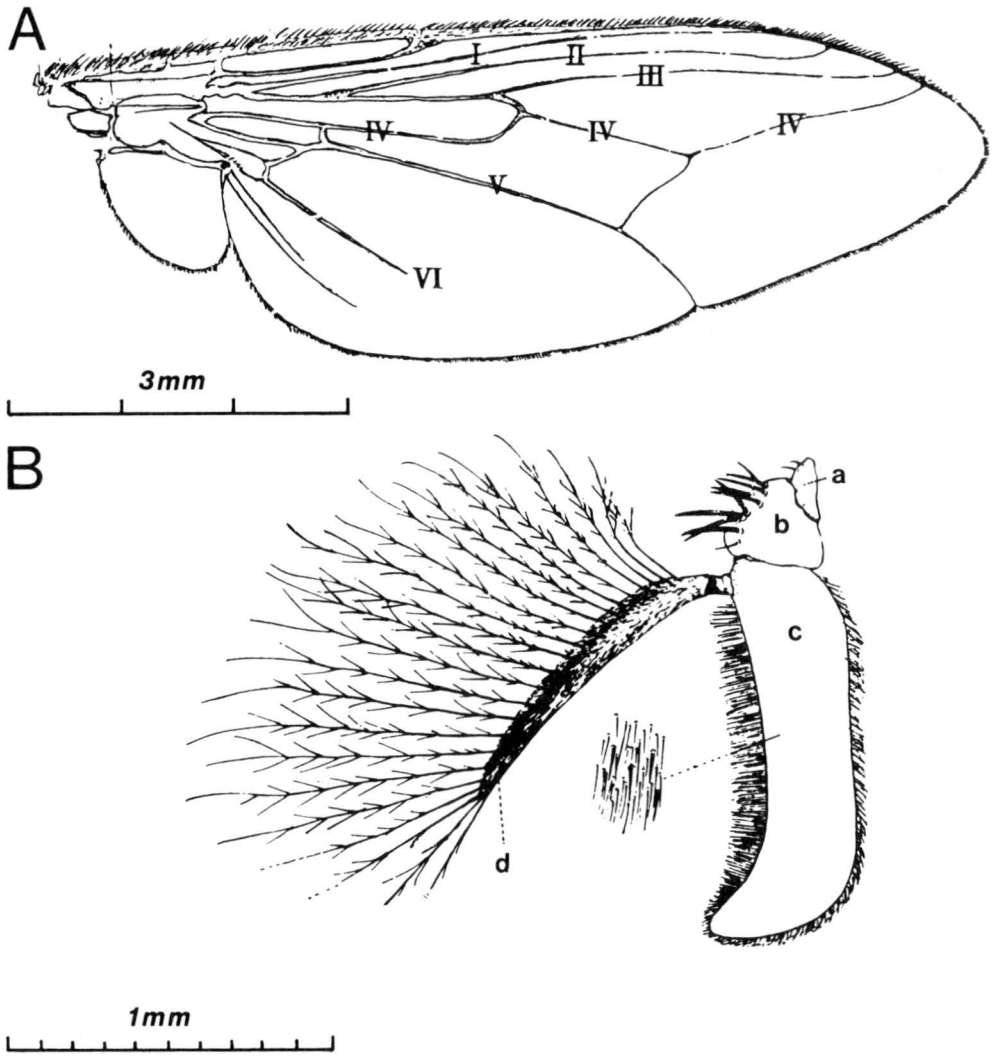
The genus *Glossina* has been included in the family Muscidae by most authorities (Newstead et al. 1924; Imms 1957), being placed with either the Stomoxyinae or in a distinct subfamily, the Glossininae. Other authorities have regarded the genus as having very uncertain affinities with the Muscidae and placed it in a monogeneric family, the Glossinidae (Brues et al. 1954; Haeselbarth et al. 1966). Still others have looked upon the Hippoboscidae (louse flies) as the nearest living relatives of the tsetse flies (Bezzi 1911; Bequaert 1954; Hennig 1965). This latter relationship has recently been examined in detail and extended to include the Gasterophilidae (botflies).

Largely on the basis of evidence from wing venation, location of abdominal spiracles, features of sperm storage in the male, and methods of sperm transfer, Pollock (1971) has grouped all three families within his superfamily Gasterophiloidae. Following up these observations, Pollock (1973) described homologous features of the genitalia and segmentation of the male abdomen of *Gasterophilus* and *Glossina*. He also provided a list of features of the inferred basic gasterophilid stock that gave rise to the extant Gasterophilidae (by loss of adult mouthparts), the Glossinidae (by evolution of adenotrophic viviparity), and the Hippoboscidae (by evolution of adenotrophic viviparity and ectoparasitism).

Accounts of various aspects of the systematics of *Glossina* are not only found in the basic work of Newstead et al. (1924) but also in Hegh (1929), Patton (1934, 1936), Zumpt (1936), Gaschen (1945), Machado (1954, 1959, 1970), and Potts (1970a, 1973).

Divisions of the Genus *Glossina*

There is general agreement that the genus *Glossina* is logically divisible into



Diagnostic features of the genus Glossina. A. The wing. The six longitudinal veins are indicated by Roman numerals. The characteristic "hatchet cell" is enclosed by veins IV and V and two cross veins. B. The antenna. Three segments (a, b, c) are present; the arista (d) has hairs that have secondary branches (courtesy Liverpool School of Tropical Medicine).

three well-marked groups (Newstead 1911). Some (Zumpt 1936; Haeselbarth et al. 1966) have given the groups the status of subgenera. The three groups are clearly distinguishable on the basis of differences of the male and female genital armature. Newstead et al. (1924) defined the groups as follows:

Group 1. The *fusca* group (or subgenus *Austenina*)

"In the males of the species included in this group, the superior claspers are quite free, there being no membrane stretching between them; the distal extremities of these appendages have either a single, large and bluntly-pointed, tooth-like extension, or they are bluntly bidentate; the harpes in all cases being markedly different in structure.

The external armature in the females consists of five plates — one pair dorsal, one pair lateral, and a single median sternal one, medio-dorsal plate absent. Internally the signum is generally well developed."

Group 2. The *palpalis* group (or subgenus *Nemorhina*)

"In the members of this group the superior claspers of the males are connected by a thin membrane which is deeply divided medially; in all cases the distal extremities of the claspers are quite free and widely separated but produced into a single more or less falciform or tooth-like process.

The external armature of the females consists of six plates. In addition to those present in Group 1, there is a small mediodorsal plate. A signum is never present."

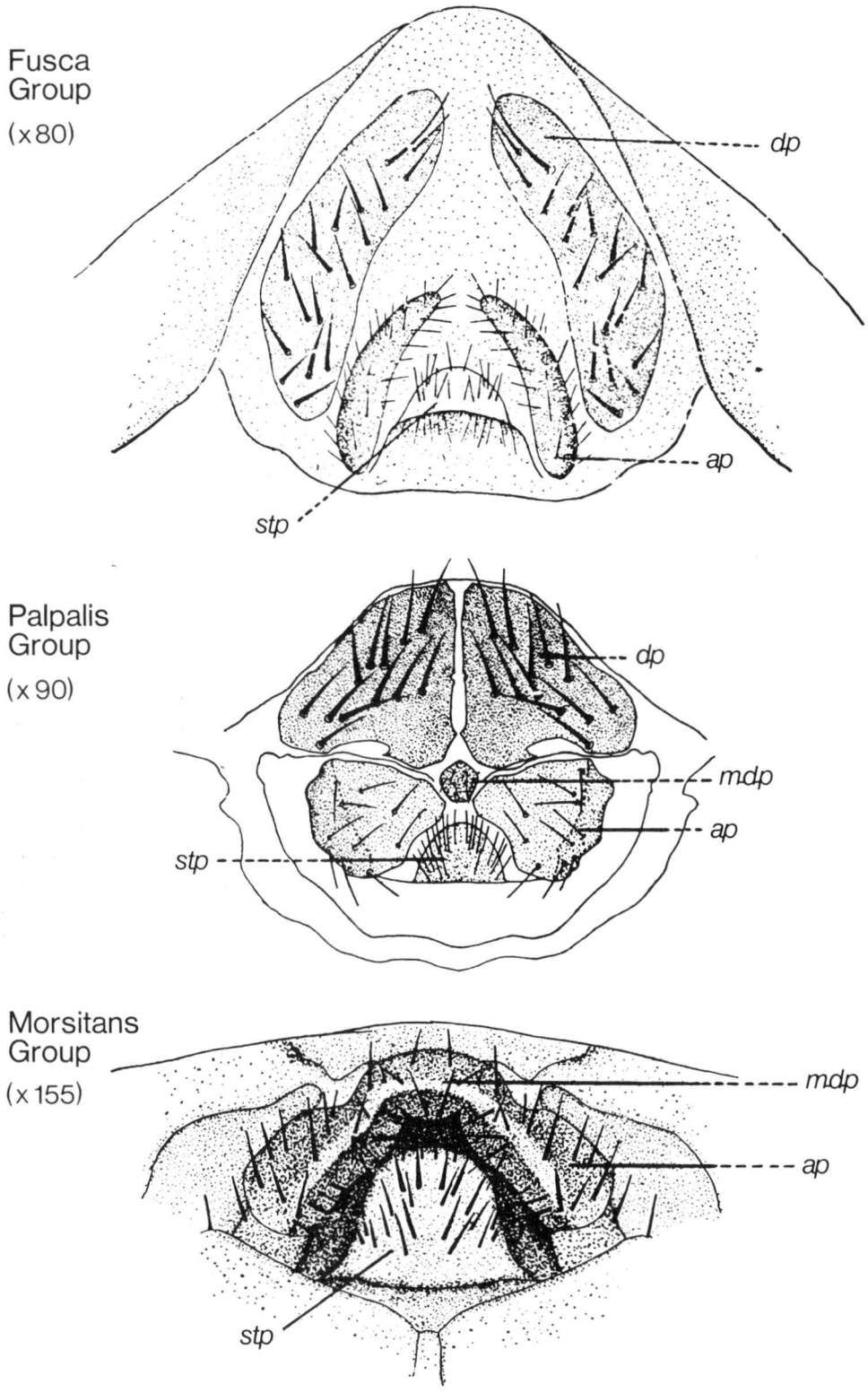
Group 3. The *morsitans* group (or subgenus *Glossina s. str.*)

"In the members of this group, the superior claspers are completely united by a membrane and they are also fused medially at the distal extremity. . .their shape somewhat resembling the scapula of a mammal in miniature, and they are altogether much more complicated structures than those in either of the preceding groups. The blunt tooth-like extension on the distal margin of the superior claspers, which is most evident in *G. morsitans* and its race *submorsitans*, and also in *G. swynnertoni*, is homologous with the terminal tooth of the superior claspers common to the species included in Groups 1 and 2.

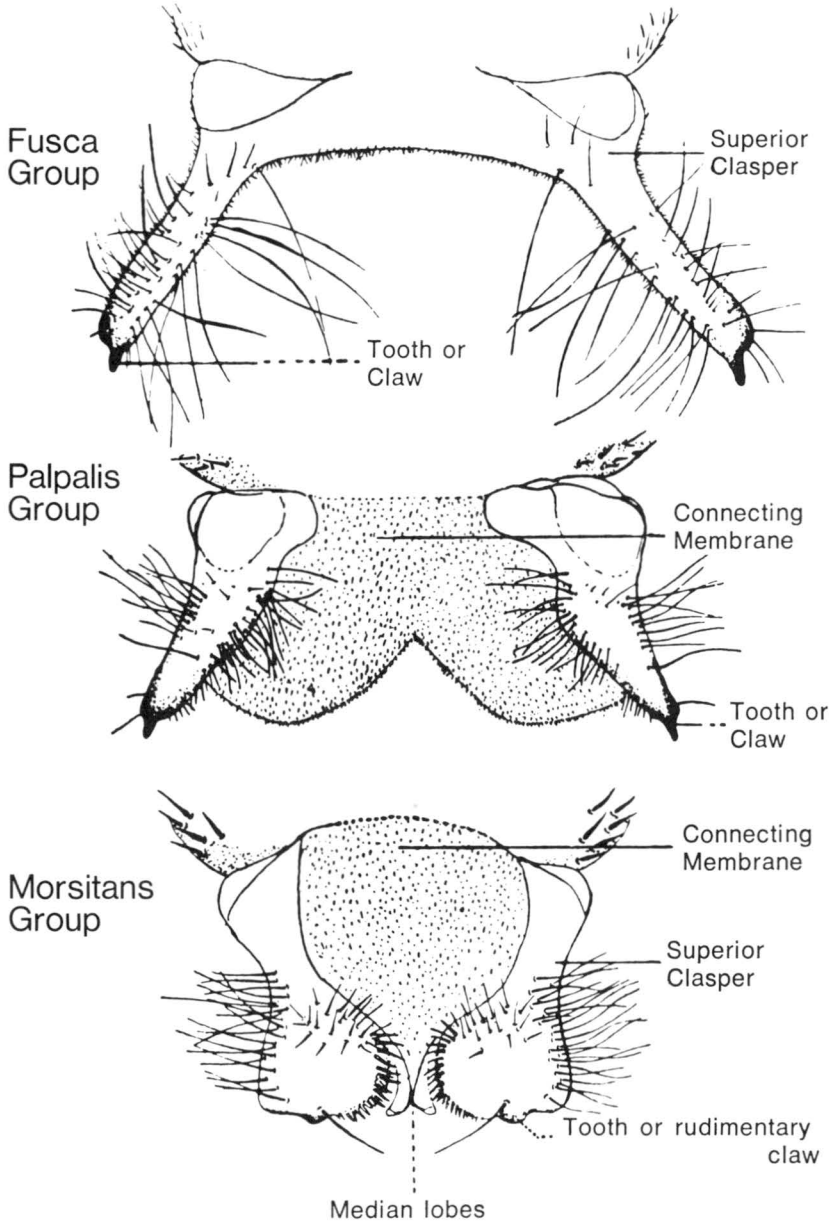
The external armature of the females consists of a pair of fused anal plates and a median sternal plate. Dorsal plates are generally absent, but occur in a reduced form in *G. austeni*. There is no signum in any of the species."

Typical forms of the male superior claspers and of the female external genital armature in the three groups are illustrated.

The division of the genus into three groups on morphological grounds has recently been confirmed by studies on the karyotypes of some of the species of *Glossina* (Itard 1966, 1970, 1971a; Hulley 1968; Riordan 1968; Baldry 1970; Maudlin 1970). Relevant literature has been reviewed by Itard (1973). Chromosome numbers have been determined for only two species of the *fusca* group, *G. fusca congolensis* ($2n = 22$) and *G. brevipalpis* ($2n = 16$). It seems that relatively large numbers of chromosomes may prove to be typical for the *fusca* group. Rather more data are available for the other two groups of the genus. In the *palpalis* group, *G. fuscipes fuscipes*, *G. palpalis palpalis*, and *G. tachinoides* have been studied — in all cases $2n = 6$. In the *morsitans* group, those species that have been investigated (*G. morsitans morsitans*, *G. m. centralis*, *G. swynnertoni*, *G. pallidipes*, *G. longipalpis*, and *G. austeni*) all have a basic number of $2n = 6$ large chromosomes, usually with the addition of a variable number (up to $2n = 8$) of smaller chromosomes. The number of smaller chromosomes can vary (both between species and between individuals of a single species/subspecies) without any apparent deleterious effect. These smaller chromosomes may appro-



Diagrammatic representation of typical forms of the external genital armature of female Glossina in the three groups into which the genus is divided: ap = anal plate; dp = dorsal plate; mdp = medio-dorsal plate; and stp = sternal plate (courtesy Liverpool School of Tropical Medicine).



Diagrammatic representation of typical forms of the superior claspers of Glossina in the three groups into which the genus is divided (courtesy Liverpool School of Tropical Medicine).

priately be regarded as supernumerary or B chromosomes (Itard 1970; Southern and Pell 1973). The discovery of polytene chromosomes in *Glossina* (Burchard and Baldry 1970; Haring 1970; Riordan 1970) has been followed by the construction of cytological maps for *G. m. morsitans* and *G. austeni* (Southern et al. 1973; Southern and Pell 1974). Further detailed studies on other species/subspecies in future should allow a deeper insight into the relationships between the various taxa of *Glossina*.

In addition to the morphological and now karyotypic evidence for the division of *Glossina* into three groups, it has long been known that this division of the genus is in the main confirmed by the habitat preferences of the various species.

The species of the *fusca* group are typically inhabitants of the great rain forests of west and west-central Africa or of the generally drier forest outliers, often associated with streams and rivers in the savannah, surrounding these forests. The distribution of the *fusca* group is much fragmented, the activities of man (primarily the felling of forest vegetation for farming purposes, and the hunting and driving away of the forest animals on which the flies feed) having rendered large areas unsuitable as habitats for these species of *Glossina*. Two species are atypical in that they occur in east Africa in still drier habitats than those species found to the west. The habitats occupied by *G. brevipalpis* (evergreen thickets and other relatively moist situations) are much closer to the humid forest habitats occupied by *G. longipennis*. This, the second atypical species of the *fusca* complex, is found under drier conditions than any other tsetse.

The *palpalis* group are also basically forest-dwellers. However, most species tend to be primarily associated with watercourses and lakeside environments. Additionally, the most important species, *G. palpalis*, *G. fuscipes*, and *G. tachinoides*, extend linearly far out into the savannah woodlands surrounding the areas of forest vegetation, occupying the thicker vegetation fringing streams, rivers, and (particularly in the case of *G. fuscipes* in east Africa) lakeshores. No species of the *palpalis* group occurs along the river systems draining into the Indian Ocean.

The species of the *morsitans* group occur mainly in the savannah woodlands surrounding the extensive forest areas of west and west-central Africa. To the north and southwest their distribution is limited by deserts, to the southeast by cold winters, and to the east by the sea. In economic terms the members of the *morsitans* group (particularly the various subspecies of *G. morsitans* itself) far outweigh in importance all the other species of *Glossina*. They occupy vast areas of potential cattle grazing country.

The Species and Subspecies of *Glossina*

The genus *Glossina* is currently considered by most authorities to include 4 extinct species and 22 extant ones. A limited number of subspecies are recognized. Table 1 (Jordan 1974b) lists the living members of the genus. Studies by Machado (1954, 1959, 1970) have contributed greatly to putting the systematics of *Glossina* onto a sound footing, particularly in clarifying the formerly confused terminology of the *palpalis* group and of the subdivisions of *G. morsitans*. Keys for the identification of the species listed in Table 1 were given by Potts (1973), who also illustrated the diagnostic features (particularly of the male and female genital armature) of the various species.

Machado (1959) studied the affinities of the species of the *fusca* group. He recognized one closely related subgroup of species comprising *G. fusca, G. haningtoni, G. nashi, G. tabaniformis, G. schwetzi, G. vanhoofi*, and *G. fuscipleuris*, with a generally western or central African distribution. *G. brevipalpis* and *G. longipennis*, although showing marked differences from one another, were considered to comprise a second, eastern subgroup. The position

Table 1. The genus *Glossina* (from Jordan 1974b).

fusca group	*palpalis* group	*morsitans* group
G. fusca	*G. palpalis*	*G. longipalpis* Wiedemann 1830
fusca (Walker) 1849	*palpalis* (Robineau-Desvoidy) 1830	*G. morsitans*
congolensis Newstead & Evans 1921	*gambiensis* Vanderplank 1949	*morsitans* Westwood 1850
G. tabaniformis Westwood 1850	*G. tachinoides* Westwood 1850	*submorsitans* Newstead 1910[a]
G. longipennis Cortie 1895	*G. pallicera*	*centralis* Machado 1970
G. brevipalpis Newstead 1910	*pallicera* Bigot 1891	*G. pallidipes* Austen 1903
G. nigrofusca	*newsteadi* Austen 1929	*G. austeni* Newstead 1912
nigrofusca Newstead 1910	*G. fuscipes*	*G. swynnertoni* Austen 1923
hopkinsi Van Emden 1944	*fuscipes* Newstead 1910	
G. fuscipleuris Austen 1911	*martinii* Zumpt 1933	
G. medicorum Austen 1911	*quanzensis* Pires 1948	
G. severini Newstead 1913	*G. caliginea* Austen 1911	
G. schwetzi Newstead & Evans 1921		
G. haningtoni Newstead & Evans 1922		
G. vanhoofi Henrard 1952		
G. nashi Potts 1955		

[a] An eastern form, *ugandensis* Vanderplank 1949, of this subspecies is recognized by Machado (1970).

of the remaining three species is uncertain. However, *G. nigrofusca* was considered to be intermediate between the two subgroups, *G. medicorum* to be rather more closely related to the first subgroup, and *G. severini* (showing a mixture of features) to be difficult to place.

The species of the *fusca* group have been studied less than those of the *palpalis* and *morsitans* groups. The possibilities for the existence of geographically separated, morphologically distinct races or subspecies have not been closely examined. Nevertheless, two species have been split into subspecies on geographical and morphological grounds. *G. n. nigrofusca,* which occurs in west Africa, is distinguishable from *G. n. hopkinsi,* which is present from Chad southeast to Uganda, by differences in the shape and pilosity of the antennae. The male and female genitalia of the two subspecies are apparently identical (Van Emden 1944; Machado 1959). Two subspecies of *G. fusca* are also recognized (Machado 1959; Le Berre and Itard 1960; Jordan 1965). *G. f. fusca* is a macrophallic form. It is restricted to the forest regions west of the Togo-Benin savannah gap. *G. f. congolensis* is a microphallic form that occurs east of the Togo-Benin savannah gap (although a few specimens have been recorded from Ghana, west of the gap) and southwards around the edge of the central African rain forest to Angola and southern Zaïre. Machado (1959) recognized a geographical cline within *G. f. congolensis* but considered this to be of less significance than the difference between *G. f. fusca* and *G. f. congolensis.*

The *palpalis* group is a much more homogeneous assemblage of species than is the *fusca* complex. The nomenclature of the *palpalis* group followed here is that proposed by Machado (1954). *G. fuscipes* has formerly been considered both as a distinct species and as a subspecies of *G. palpalis.* Machado (1954) reestablished the specific status of *G. fuscipes* originally proposed by Newstead (1910). Three of the five species in the group have been further divided into a number of subspecies. Two subspecies of *G. palpalis* are recognized: (1) *G. p. gambiensis* to the west of Togo-Benin; and (2) *G. p. palpalis* east to Cameroon and then extending south, along the western seaboard of Africa, to Angola. Intermediate forms frequently occur in an extensive zone of overlap of the two subspecies. Identification of the two forms is primarily based on differences in features of the male and female genital armature. *G. fuscipes* is divided into three subspecies that together occupy the block of west-central African rain forest and extend out-

wards along watercourses into the surrounding savannahs. Their distribution is extended to the east to include the shores and river systems associated with some of the great lakes of east Africa, particularly Lakes Victoria and Tanganyika. *G. f. fuscipes* occurs in the northern part of this distribution, ranging from Cameroon in the west, to Kenya in the east, and to Angola and central Zaïre in the south. *G. f. quanzensis* is found in the southwest of the species' area of distribution, primarily in northern Angola and southwest Zaïre.

G. f. martinii occurs in the southeast of this area, primarily in southeast Zaïre but extending into western Tanzania and northern Zambia. Only limited zones of overlap occur between the three subspecies. The subspecies are distinguished by characteristics of the male and female genitalia. Although some variation occurs within the subspecies, no incontrovertible transitional forms have been described (Machado 1954). *G. pallicera* is also divided into two subspieces. *G. p. pallicera* occurs in rain forest from Sierra Leone to Cameroon. It is separated geographically from *G. p. newsteadi*, which occurs within the extensive rain forests of west-central Africa. The two species are distinguished by characteristics of the genitalia and the extent of pilosity of the third segment of the antennae.

Five species are recognized within the *morsitans* groups of *Glossina*. However, in view of the close relationship between certain of the species, it is perhaps realistic to consider the group to comprise two species-complexes (*G. longipalpis* and *G. pallidipes* forming one complex, and *G. swynnertoni* and the various subspecies of *G. morsitans* the other). One somewhat aberrant member, *G. austeni*, is undoubtedly very closely related to *G. longipalpis* and *G. pallidipes*. They can only be separated on morphological grounds by a few fine details including the coloration of the last tarsal segment of the foreleg and minor variations in the male genitalia. Haeselbarth et al. (1966) considered *G. pallidipes* to be a subspecies of *G. longipalpis*. Here, though, we follow Machado (1965, 1966) and consider the two species as distinct until such time as a thorough reexamination of their systematics has been undertaken. The distributions of the two species do not overlap. The western one, *G. longipalpis*, is separated from the eastern *G. pallidipes*, by about 1000 km. *G. longipalpis*, limited to a relatively narrow band of savannah vegetation north of the large blocks of west and west-central African rain forest, is closely associated with thickets. *G. pallidipes* (East Africa) is also closely associated with thickets but extends from Ethiopia in the north to Mozambique in the south. Many of its habitats are very much drier than any of those occupied by *G. longipalpis*. This suggests that there may well be physiological differences between the two species.

A variety of minor taxa of *G. morsitans* have been described. However, it is proposed to follow the revision of Machado (1970), and to recognize only three subspecies (referred to by him as "races géographiques majeures"). Because of confusion over the locality of origin of the original type specimen described by Westwood (1850), the subspecies formerly referred to as *G. m. orientalis* Vanderplank becomes *G. m. morsitans* Westwood; and that referred to by Vanderplank (1949) and various subsequent authors as *G. m. morsitans* Westwood becomes *G. m. centralis* Machado. An eastern form (*ugandensis* Vanderplank) of *G. m. submorsitans* is recognized. *G. swynnertoni* is considered as a separate species, although closely related to *G. morsitans* (Machado 1966). The three subspecies of *G. morsitans* and *G. swynnertoni* can be readily distinguished by features of the male genitalia. The subspecies of *G. morsitans* are generally separated from one another geographically. *G. m. submorsitans* occurs in much of the savannah woodland north of the blocks of rain forest, extending from Senegal in the west to Ethiopia in the east. *G. m. centralis* occurs mainly in the savannahs east and south of the west-central African rain forest, although it is also found to the south-

west of the forest in Angola. *G. m. morsitans* occurs to the east of *G. m. centralis*. Machado (personal communication) has distinguished a zone of overlap of *G. m. morsitans* and *G. m. centralis* in Zambia, whereas there is some overlap of *G. m. centralis* and *G. swynnertoni* (which has a very restricted distribution) in Tanzania. Some of the possible combinations of crosses between the subspecies of *G. morsitans* and *G. swynnertoni* have been achieved in the laboratory (Vanderplank 1947, 1948; Curtis 1972). Most degrees of resulting hybrid sterility confirm the taxonomic categories recognized by Machado (1966, 1970). Curtis (1972), though, concluded that *G. swynnertoni* is about as genetically compatible with the subspecies of *G. morsitans* as they are with one another.

The Phylogeny of *Glossina*

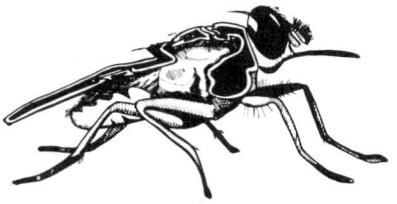
The genus *Glossina* is evidently an ancient one. Fossil examples attributed to four species have been recorded from sedimentary shales in Colorado, USA (Cockerell 1918). Although there is some doubt as to the precise age of these deposits, they are generally attributed to the Oligocene. By this time the genus was already displaying considerable variation, at least with respect to size. The wing lengths of *G. oligocena* and *G. osborni* were 16 and 7 mm, respectively. Unfortunately, the structural details of the genitalia of the fossil species are unknown.

A number of authorities have speculated on the relationships between the fossil and extant species of *Glossina*, and on the likely distribution of the genus in earlier geological periods. Much of the current opinion on this subject was reviewed and analyzed by Ford (1970). Evens (1953) postulated that the fossil species were closely related to the present day species of the *fusca* group. Machado (1959) disputed this, submitting that the paleontological evidence reveals no more than that the genus has shown considerable variation for a long period of time, and that it previously extended beyond its present range.

A number of authorities, like Evens (1953) — although basing their conclusions on other criteria — have suggested that the living members of the *fusca* group show more "primitive" features than the species of the *palpalis* and *morsitans* groups. Newstead et al. (1924) considered that the superior claspers of the male become progressively more complicated through the groups *fusca* — *palpalis* — *morsitans*. Bursell (1958) also considered the modern *fusca* group to be the most primitive. He postulated that the ancestral *Glossina* inhabited a humid forest environment, as do most members of the *fusca* group of today. Bursell also suggested that divergence from this ancestral type was associated with increased water-proofing of the puparia, resulting in speciation and the evolution of forms able to survive in drier environments. He saw evolution as progressing from a predominantly hydrophilic *fusca* group (via an intermediate *palpalis* group) to a predominantly xerophyllic *morsitans* group. *G. longipennis* (a member of the *fusca* group and an inhabitant of extremely dry areas) and *G. austeni* (a member of the *morsitans* group and an inhabitant of moist environments) were considered as examples of reversion to drier or wetter environments after the adoption of a basic habitat by the group to which they belong.

Machado (1959) questioned these widely held views. While agreeing that the superior claspers appear to become progressively more complex from the *fusca* group to the *morsitans* group, he pointed out that the complicated harpes of many species of the former can hardly be considered as a primitive feature. Recently Pollock (1973) has taken the argument a step further. He suggested that the present-day species of the *morsitans* group should be considered as possessing the most primitive features, and those of the *fusca* group, the most specialized ones. He based this conclusion on evidence that species of the

morsitans group are morphologically much closer to *Gasterophilus* than species of the *fusca* group. Such a conclusion is only relevant if one accepts the postulated common origin of the extant Gasterophilidae and Glossinidae in a basic gasterophilid stock. — A.M. Jordan.



Control Methods

Twenty years ago, an attempt to classify methods of tsetse control included: direct attack; starvation; habitat modification, and autosterilization. Direct attack included controls by: (1) parasites and predators; (2) hand-catching and traps; and (3) insecticides. Under the heading starvation, it was noted that it was uncertain whether game (wildlife) elimination acts solely by inducing starvation. It is still uncertain. Under habitat modification were noted (1) sheer and (2) partial bush clearing, and also (3) human occupation. Finally, under the title autosterilization came (1) hybridization and (2) male sterilization by gamma rays (ISCTR 1958). The two latter methods are now called autocidal or genetic control. Two decades ago, they evoked little enthusiasm. Today they absorb a large part of research expenditure on tsetse control by the aid-nations. For a recent revision of the above classification under the headings of direct and indirect attack, see Potts (1970c).

No serious appraisal of trypanosomiasis control techniques, of which tsetse abatement is one, can be made outside the context of African cultural and economic development. Of immediate relevance to such an approach, of course, are the risks of disruptive epidemics of sleeping sickness such as have occurred in the last 15 years around the rim of the Zaïre basin and (on a much smaller scale, but disturbingly widespread) elsewhere.

The African trypanosomiases are principally remarkable for the vast area in which their presence inhibits various sorts of rural development. It has to be emphasized, however, that these diseases do not comprise the only obstacles to the exploitation of the ten million or so square kilometres infested by *Glossina*. Throughout much of the area, intensive cultivation (except on a relatively small scale) has been prevented by extreme poverty in soil nutrients; combined on the one hand, with unreliability of rainfall and, on the other, with inadequate or patchily distributed groundwater. It is not just with respect to African peasant agriculture that this is true. A number of expensive failures of modern technology in the last quarter-century also bear witness to the fact that control or eradication of trypanosomiasis is not automatically followed by economic improvement. This may be emphasized by reference to costs.

In Uganda, from 1947 to 1955, the cost of freeing 18 500 km^2 from tsetse flies was 7.4 Uganda shillings (Ushs) per hectare. Another 6600 km^2 reclaimed in the next 11 years cost 63.0 Ushs/hectare. Finally in the 5 years up to 1971 costs rose to 84.7 Ushs/-ha, when 5900 ha were reclaimed (Jahnke 1973). The increases in cost were not entirely due to inflation. They also reflected the need for special measures, never fully successful, to prevent reinfestation. Another cause of cost-increase springs from the fact that as the area treated is enlarged, so the distances from centres of supply also increase. Clearly, there is a need for tsetse control to proceed at a rate geared to the rate of economic expansion and the establishment of new rural industry.

This was recognized, in the early years of political independence, by the provision of funds from the European Economic Community (EEC) for the reclamation and development of the Bugesera region of

Rwanda. Twenty-five years previously, this region had been invaded by *Glossina morsitans* carrying both animal and human trypanosomiasis (due to *Trypanosoma rhodesiense*). At a cost of $1 US per hectare a 98% control was achieved over the tsetse flies by discriminative aerial application of Telodrin to only 13% of the infested area (Buyckx 1964, 1965). The important point here is that the funding of the *Glossina* control work accounted for only 1.5% of the allocation for development of the area as a whole. For most of the deciduous woodlands and wooded savannah infested by *G. morsitans*, expenditure of comparable proportion for tsetse control:total development costs, is still probably correct. However, it may well be that total development expenditure may have to be spread over a period so long that the initial and essential attack on the trypanosomiases is forgotten by the time full productivity is achieved.

The problems of world population growth and resource conservation compel one to look at the biological control of *Glossina* not only in relation to other methods, but also as a technique for ecosystem management. The ecosystems affected by trypanosomiasis can be regarded as comprising five groups of organisms: (1) the large wild vertebrate fauna, supporting as their own internal parasites (2) the trypanosomes pathogenic to (3) the larger domestic livestock and (4) man himself. The tsetse flies (5) sustain transmission of the trypanosomes between the other four groups. Each of the five is, itself, a system of great complexity — ecological, physiological, behavioural, and genetic. Much research immediately relevant to adequately planned control still remains to be done in each of these fields.

Any successful control operation, by any means not involving wasteful destruction of natural resources (except, perhaps, wholesale bush clearing preliminary to intensive cultivation), must be planned on a basis resulting from study of the distribution, behaviour, and densities of the *Glossina* populations throughout their environment. Such work owes a great deal to early field investigations carried out in Tanzania between 1925 and 1950, under the particular influence of C.F.M. Swynnerton, to whom many of the relationships outlined above were familiar. Much influenced by the American plant ecologist F.E. Clements, Swynnerton conceived the natural biome as having a characteristic structure, furnishing the physical environment in which *Glossina* spp. find shelter from extremes of climate, perching places from which to observe the arrival of host animals, and breeding sites where females larviposit.

An exhaustive account of the hypotheses developed from 1930 onwards to explain the relationship between the observed responses of a *G. morsitans* population to the human observer and the vegetation patterns related to soil catenas associated with drainage systems, would require several chapters. It is sufficient to note that it eventually proved that relatively dense populations of this tsetse (in which the proportion of females caught is very small) tended to be associated, throughout vast areas of *Brachystegia–Julbernardia* woodland with ecotonal vegetation bordering drainage lines. These often ran into seasonal swamps variously known as dambos, mbugas, vleis, or mandas. The low percentage of females caught indicated that these peripheral ecotones were the zones in which breeding tended to be concentrated and the tsetse population as a whole was well nourished. Felling of arboreal vegetation in such ecotonal zones was generally followed by a pronounced decline in the tsetse catch, in some cases to zero. This method of discriminative clearing usually involved the destruction of about 5% of the total tree cover. It commended itself to Swynnerton's team because it appeared to minimize alteration in the natural ecosystem as well as avoiding any attack on the wildlife.

The true explanation of the low female percentage in the catch made by men using nets has only recently become clear

from the work of Vale (1974) using simple electrical devices of great ingenuity. The theory of discriminative clearing was, in many respects, faulty. However, it formed the basis for many useful control schemes at a time when large scale wildlife slaughter was the only alternative. Aerial photography began to be used for planning control measures shortly before the synthetic organic pesticides came into use. The mapping of vegetation patterns to show the so-called "true habitat" of *G. morsitans* and related species such as *G. swynnertoni* and *G. pallidipes* was successfully taken over by the newer techniques (for example, as in the Rwanda operations described above). It is to be emphasized that any control method involving the introduction of biocontrol agents (whether pathogens, parasites, or predators, or autocidal agents such as artificially sterilized males) will necessarily make extensive use of the planning techniques developed for discriminative clearing and later refined for insecticide application.

A general recipe for eliminating *Glossina* cannot be produced. The elements in the habitat rendering populations of *Glossina* (of the *morsitans* and *palpalis* groups) vulnerable to attack by partial or discriminative bush clearing (Swynnerton 1936; Van den Berghe and Lambrecht 1963) differ according to the climatic zones and vegetation types in which they are situated. From the detailed analysis (Nash and Page 1953) of the microclimates used by *G. p. palpalis* populations in the Nigerian-northern Guinea zone it thus seems clear that the effectiveness of the partial felling of riverine trees advocated by these authors must have been due to the destruction of the flies' ecoclimate. Nevertheless, the recent studies by Challier (1973, 1975) reveal that the population density of *G. p. gambiensis* is controlled in nature by a complex of diverse factors acting in such a way that the tsetse population is always in a state of unstable equilibrium.

On the other hand, the probable cause of *G. m. centralis* population decline after felling of ecotonal woodlands in the western flybelt of Tanzania now seems to have lain in its interference with feeding behaviour rather than in its destruction of the ecoclimate. It has long been known that warthogs (*Phacochoerus aethiopicus*) generally supply from 30 to 50% of the blood meals of *G. morsitans* in these areas. Warthog distribution is linked to the distribution of burrows made by antbears or aardvarks (*Orycteropus afer*), which they use as shelters. The distribution of antbears is closely correlated with the distribution of large mound-building termites (*Macrotermes* spp. and associated Isoptera) in seeking which the antbears construct their burrows. Termitaria may be found in a variety of sites throughout the general woodland. However, they tend to be concentrated in a band 50–100 m wide along drainage systems and around the seasonal swamps, which support a characteristic evergreen flora of trees and shrubs, giving the ecotone its principal characteristic.

Especially in the dry weather (when the concentration of tsetses is greatest in the ecotones), warthogs feed upon the roots of grasses, such as *Hyparrhenia* spp., flourishing in the seasonal swamps. Bush felling in this zone may therefore destroy the warthog habitat, so interfering with both the feeding and mating patterns of tsetse. This conclusion derives support from observations that the decline in density of the *G. morsitans* population is accompanied by an increase in the proportion of females taken in net catches. This is indicative of a generally poor state of nutrition. Further south, under a somewhat more arid climate in the Zambezi river valley, Pilson and Pilson (1967) showed that antbear burrows have an important function as refuges from the dangerously high midday temperatures of the mopane (*Colophospermum*) woodlands. Indeed, without them, and other holes, *G. m. morsitans* would be unable to survive in the leafless woodland at the height of the hot season. It is worth noting that the antbear (which being nocturnal escapes attack by *Glossina*) dies if artificially in-

fected with trypanosomes (Ashcroft 1959).

Attack on *G. morsitans* by discriminative clearing or selectively applied insecticide in environments of the types described, have not always been successful. Where there has been success, doubts have remained about whether this has been due to the specifically anti-tsetse operations or to the actions of new settlers in further expelling or disturbing the wild host animals. Often, however, where initial success has been incomplete, the continuation of surveys and use of the control technique against residual pockets of tsetse infestation has had to be continued over several seasons.

The problems of planning surveillance and of application of whatever control technique is decided upon, become simpler as one approaches the hotter and drier climates limiting the continental distribution of the genus. The greatest successes (in terms of area) achieved without slaughter of wildlife, have been located in the Sudan and northern Guinean zones of northern Nigeria and in the Sabi-Lundi river basin of Rhodesia and Mozambique.

In Nigeria *G. m. submorsitans, G. p. palpalis,* and *G. tachinoides* may occupy the same flybelt. The attack on them (by selective application of 3.75% DDT in water) began in 1955, at first separately (MacLennan 1958; MacLennan and Kirby 1958) and later simultaneously (MacLennan and Aitchison 1963). This work still continues. By 1966 an area of 32 000 km^2 had been successfully treated. By 1971 the greater part of a 94 000 km^2 block additional to the first, had been covered by the extermination units (MacLennan and Na'isa 1971). It is too soon to know whether these operations have been completely effective. One advantage peculiar to Nigeria is that its *G. morsitans* belts are much divided. In 1956 a map published by MacLennan (1958) showed 31 discrete belts. It must be noted, however, that while the control operations have been in progress, several of these belts have expanded and coalesced.

The southern scheme, on the contrary, had to deal with an expansion of a flybelt stretching northward for some 2000 km (broken only by the narrow gap of the Zambezi River) almost to the border of Kenya. It was chiefly remarkable in calling for, and receiving, the collaboration of three governments and three control staffs; for although the tsetses were located in Rhodesia and Mozambique, the principal threat was to the valley of the Limpopo River and northern parts of South Africa — including the Kruger National Park, which had become free from tsetse following the rinderpest panzootic at the end of the 19th century (Ford 1971). Robertson et al. (1972) reported the successful conclusion of an insecticide operation covering 10 950 km^2 during nine seasons of work, at a cost of US $0.83/ha of land infested by *G. m. morsitans* and *G. pallidipes*. In general the cost of tsetse control with insecticides is very much cheaper than the older methods of bush clearing, whether by manual labour or mechanically.

One result of the advent of synthetic pesticides was a great enlargement of the field of research into *Glossina* behaviour. Studies on the resting habits of tsetses were begun by Nash and Davey (1950) as a means of finding species which do not readily approach man. Somewhat similarly, Isherwood (1957) studied *G. swynnertoni* at rest, in an attempt to elucidate the low response of its females to man. The relevance of such studies to insecticidal control was realized as soon as formulations and application techniques were developed to take advantage of the residual properties of some of these chemicals. (Initially, the use of smokes and fogs did not appear to demand knowledge of the insects' whereabouts, penetration of the whole vegetation space seeming to be of the first importance — Burnett (1970) reviews these methods of control.)

With the introduction of residual insecticides such as DDT in aqueous suspension, and dieldrin, information on resting sites (including seasonal and circadian variations) became one of the essential preliminaries to control work. Thus Davies

Pesticide residues are selectively applied to the boles of isolated trees in the Sudan zone of Nigeria, Jamaari River 1963 (John Ford).

(1964) describes measures taken, in successive dry seasons from 1955 to 1964, against *G. m. submorsitans* and *G. tachinoides* on rivers draining from northeast Nigeria into Lake Chad. The technique finally developed, partly through study of resting sites, partly empirically, eventually involved application of the insecticide to the trunks of trees of more than 23 cm in diameter growing in heavy shade on the riverine flood plain. Where *G. morsitans* was present in high density, these trunks were sprayed to a height of 1.5 m, but where *G. tachinoides* was found alone or accompanied by only low density *G. morsitans* it was possible to confine spraying of these tree trunks to the first 0.6 m above ground. (For studies of resting sites in hot, semi-arid environments near the southern limits of *Glossina* see Pilson and Leggate 1962; Pilson and Pilson 1967.)

Treament of only the larger tree trunks does not work in the woodlands of the more humid northern Guinean zone of Nigeria, where a situation rather like that described above in western Tanzania is found, *Brachystegia* spp. being replaced as dominants by the closely related *Isoberlinia* spp. Here teams of four sprayers, covering between them a swath of 75 m, move along each side of the drainage lines applying the insecticide to the undersides of branches of 5 cm or more width at heights between 1.5–3.7 m above the ground (MacLennan 1967). It will be seen that the insecticide is applied precisely in that zone which, in earlier years, would have been discriminatively cleared.

The objections first raised some years ago in North America to the widespread use of persistent pesticides to control insects injurious to food crops, have had little impact so far on their use against tsetse flies. Where concern has been expressed, it has focused more upon wildlife than upon the human inhabitants of the flybelts. Koeman et al. (1971) studied faunal changes following dieldrin spraying

Selective application of DDT to adult resting place of G. tachinoides (branches of Tamarindus indica L.), Kalamaloué Reserve, Chad (1 March 1973, M. Laird).

against *G. palpalis* and *G. tachinoides* of the Matyoro swamp (Gongola River, northeastern Nigeria). The principal finding was of a general decline in the prevalence of certain insectivorous birds 1 year after a single dieldrin application. Only one species, the white-crowned robin chat, disappeared completely. Obviously, permanent disappearance would depend upon the ability of an eliminated species to immigrate again from neighbouring populations. Although some deaths were caused by direct dieldrin poisoning, insectivorous birds also suffered because of high mortality among their insect prey. Langridge and Mugutu (1968) also studied the after-effects of dieldrin spraying. They found corpses of a number of small mammals (of which the largest was a vervet monkey) birds, snakes, and lizards. They also studied mortality in the insect population, hanging 84 cm diameter drop-nets under the vegetation to be treated. The mean daily catch of ants in the 3 days before spraying was 28.3. In the 3 days following it was 149.0, and in the last 9 days of the month after spraying (days 22 to 31 inclusive) the drop-nets yielded only a daily mean of 6.2 ants. The corresponding figures for spiders were 6.3, 87.3, and 2.5,

and for wasps 1.7, 8.0, and 0.0. Mosquitoes (mainly Culicini) formed the greater part of the total kill, and yielded 70.3 individuals for the mean daily prespraying catch, 180.3 immediately after, and 78.6 at the end of the month. This indicated that, unlike the predators and foragers, they had made a complete recovery.

The first successful eradication of a flybelt was carried out by du Toit et al. (1954). They pointed out that the tsetse parasitoid[1] *Thyridanthrax brevifacies* appeared to have been eliminated more quickly than its hosts. Therefore, they urged, insecticide applications should always be kept up until the achievement of complete eradication of *Glossina*. Rogers (1975) recently proved a density-dependent relationship between tsetse incidence and the intensity of predation by birds. He showed, too, that spider predation affected a constant proportion of the tsetse population, regardless of the latter's density. Rogers also demonstrated a density-dependent relationship between the incidence of *Glossina* puparia and predation by ants of the genus *Pheidole*.

An unsatisfactory feature of some foreign aid programs is that they are budgeted for the application of a prestated amount of insecticide. The program closes when the spraying is completed, and its results—only insofar as *Glossina* is concerned — are assessed. It is usual to ignore the effects of ecological imbalance, whether on residual populations of *Glossina* (if the program is unsuccessful) or on other organisms (if it is not). The little work so far done suggests that much more field research is needed on these wider ecological aspects of tsetse control. It is, however, necessary to note that although very large areas may be involved in these operations, the amount of insecticide that has been used in selectively applied ground sprays in semi-arid environments is comparatively small.

Cockbill (1970) thus stated that during a single year in Rhodesia, 151 metric tons of 75% DDT were dispensed at an average rate of 5 g/acre applied to the bark of large trees, to rot holes in trees, to the surfaces of fallen logs, and to holes in the ground. Environmental pollution from these sites is trivial compared with that from those general agricultural practices in which persistent pesticides are applied at rates of 0.45–0.68 kg/acre to growing crops several times in each season.

Each new insecticide or the extension of use of an insecticide into new environments involves trials of its toxicity to *Glossina* and its persistence on different surfaces (bark of local trees, leaves, soil surfaces, etc.). Also, it is necessary to study resting behaviour. It has already been noted that as one moves away from the arid borders of the *Glossina* belt towards the generally more humid equatorial regions, selectivity in insecticide treatment against the *palpalis* and *morsitans* groups becomes more difficult. Some of the problems thus created have been solved by recently developed techniques, for example the application of endosulfan in invert emulsion from helicopters (see discussion of these and other newer techniques on p. 177–186.

So far, we have been concerned only with control of the commoner species of the *morsitans* and *palpalis* groups. At one time, *fusca*-group tsetse flies were regarded as of little importance. This view is no longer tenable. Apart from their possible role in sustaining transmission of human-infective trypanosomes among wild animal reservoirs (which is suggested by some features of the Zaïre epidemics), it is evident that they cannot be ignored in the control of the bovine infections. Thus Kangwagye (1971) has pointed out that as the savannah-dwelling *Glossina* are removed, cattle are able to range more widely. They then tend to encounter forest edges, where they contact the *fusca*-group flies. This, of course, is particularly noticeable in countries like Uganda, which extend across the boundaries of rain forest as

[1] Entomophagous insects in which the nonparasitic (adult) phase is free-living.

well as savannah. In certain situations the movements of livestock may bring them close to the forest edge. At the northern end of Lake Malawi the mountainous country of the Malawi-Tanzania border carries forest and thicket infested with *G. brevipalpis*. Seasonal movement of cattle between kraals and also the habit of taking the animals into the forest periodically to drink at saline springs resulted in heavy infection. Control work begun in 1948 (preinsecticide) relied upon cutting out lower storey shrubs and lianas. This left the larger trees untouched (Mitchell and Steele 1956; Lloyd 1959). Near the border of the Central African Republic and Cameroon, cattle made contact with *G. fusca* while passing a tongue of high forest during transhumance journeys. Dry-season resting-site studies inside the forest showed that *G. fusca* rested on trees and lianas up to 40 cm in circumference. None were ever found on trunks greater than 90 cm in girth, while 96% of the flies were found between 60 and 200 cm above the ground. Application of 2% dieldrin emulsion from knapsack sprayers successfully dealt with this situation (Finelle et al. 1962). The indication of this work, that selective control of *fusca*-group tsetses may not be very difficult, is supported by the observations of Kangwagye (1971). He showed that after feeding, most Ugandan *G. fuscipleuris* rest head-downwards on shrub and sapling stems of 2.5–4 cm diameter, at heights of only 15–20 cm above the ground. A few others rest up to 60 cm above ground, only one having been caught as high as 2.7 m. The low-level perching places correspond to the low-level biting sites on a bait ox (also in western Uganda), where many of the feeding flies were taken in the hollows behind the pastern joints (Ford, unpublished). In central Nigeria, Nash and Davey (1950) found *G. medicorum* rather more widely dispersed up and down saplings mostly about 2.5 cm in diameter. These examples suggest that many, if not all, species of the *fusca* group might be susceptible to selective treatment either by clearing or by insecticides.

One of us (Ford) has often said that complete elimination of tsetse flies from Africa would be possible with techniques now in common use, provided it was worth the cost. This possibility, even when partial bush clearing is the only alternative to wildlife slaughter, was already being propounded over 30 years ago for much of the area infested with *morsitans*-group flies (Bax 1944). When, soon afterwards, DDT came on the market, an eminent chemist is reputed to have said of the tsetse problem 'Give me a fleet of bombers and I will finish the job!' However, when du Toit was provided with this opportunity using aircraft of the South African Air Force (then being phased down after World War II) to spray DDT over the *G. pallidipes*-infested wildlife reserves of Zululand, and was successful (du Toit 1947; du Toit et al. 1954), the achievement was not received enthusiastically by workers further north. It is pertinent to the subject of this book to enquire why this should have been so.

Only one objection to the extension of the South African technique to the main *Glossina* area further north was really relevant. It depended upon the fact that the Zululand *G. pallidipes* were isolated from the flybelt in Mozambique. The chances of reinfestation were thus very remote. The spraying and supplementary operations, in which there was a large experimental element, cost about £2 million. They were confined largely to three areas together totalling less than 51 720 ha. This resulted in the freeing from infection of cattle living in a surrounding area of some 18 000 km^2. Workers north of the Limpopo had to control trypanosomiasis derived from flybelts measured, not in hundreds, but in tens or even hundreds of thousands of square kilometres. Even with costs reduced to a few pence per hectare, the financial problem of dealing with, for example, the western flybelt of Tanzania (approximately 260 000 km^2) would be enormous. In any case, unless the area could be divided up (as suggested for bush clearing by Bax 1944) into blocks separated by barriers, reinfestation might occur more rapidly

from the untreated areas than the latter could be sprayed. One has also to remember that whereas the South African work benefited farmers who were making a valuable contribution to the national economy, this could hardly be said of most of the *G. morsitans* infested area further north. The well-publicized "groundnut scheme" had recently demonstrated, at a cost of many millions of pounds, that modern technology might be even less effective than African peasant agriculture in raising food productivity in those woodlands (see also Ford 1969, 1971, on the economic potential of the Zambezi valley flybelts).

Some of the arguments surrounding the Zululand scheme are still valid. However, not only have techniques of tsetse control been improved, but the economic, social, and political circumstances in which they are applied have changed even more markedly. Above all, perhaps, has been the realization that trypanosomiasis control (especially in domestic livestock) has to be regarded as only one among many items in the complex problem of resource management.

On the purely practical level, greater emphasis than formerly, is now laid upon the integration of trypanosomiasis control with the creation of the infrastructure of development. A project that may have to cover many thousands of square kilometres cannot be set in motion unless the area is supplied, beforehand, with at least the minimum requirements of living. The construction of boreholes or storage dams for water, roads, housing, landing grounds, a supply organization for food, fuel, medicines (as well as pesticides), and vehicle repair systems, are all essential preliminaries that may have to be provided in country where previously little that was man-made had existed. In the now comparatively distant past, when colonial administrators believed that tsetse control had to preceed development, these tasks tended to be performed by tsetse control departments. Eventually it was realized that such matters were the concern of all involved in rural development. Today, in a well run trypanosomiasis control scheme, all who will play a part in it are involved from the start.

The control of trypanosomiasis does not always bring entirely beneficial results. Thus, at one time, it appeared that, given good husbandry, it should be possible to raise cattle, under drug protection in tsetse-infested bush, without preliminary resort to vector control. The latter would be undertaken by farm staff progressively and whenever convenient. Such a program was instituted in a commercial ranch in Tanzania. Cattle were indeed raised successfully and good progress was made in reducing, by use of insecticides, the populations of the three species of *Glossina* present. However, what had not been foreseen was that one could not introduce a large biomass of domestic herbivores into an ecosystem already stocked with wild animals, without profoundly altering its whole ecology. Grass, in the tsetse-infested wooded savannah, is sustained by burning, which prevents the proliferation of woody plants. Overconsumption of grass reduces the effectiveness of the fire, so that tree and shrub seedlings that normally would be destroyed, survive. As they grow, so the area of pasture diminishes. Some bush clearing had, in fact, been written into the scheme we have mentioned, principally to prevent reinfestation by tsetse flies once they had been eliminated; but it was not enough to equal the speed at which trees and shrubs invaded the over-burdened grass (Ford and Blaser 1971).

At present, a debate is in progress about the scale on which development projects, including disease control, should be undertaken. On the one hand there are the advocates of campaigns that would apply proven techniques, if not to continents at least to whole groups of countries, to eliminate a disease or provide a service in a single operation. In the early sixties, aid-giving countries tended to believe that this was the right approach — massive injection of capital was held to be what was most needed. Against that, many now believe in projects based on intensive "small

area research" in which the study of the ecological relationships of agents affecting population increase and decrease are balanced against resource potential in the light of the social needs of relatively small communities. The Mkwaja ranch described above might be regarded as a small project of commercial enterprise for which the preliminary research turned out to be inadequate. At all events, as Jordan (1976) has stressed, rational plans for land development should be integrated with future tsetse control campaigns to guard against the under- or over-exploitation of land cleared of *Glossina.*

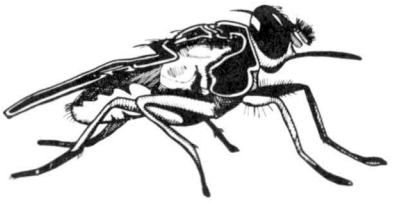
In truth, though, every project creates new problems. A large-scale example seems to be provided by the terrible famine which, in the last decade, has afflicted the populations of both man and his domestic animals, right across subSaharan Africa from Mali to Somalia. While certainly initiated by a prolonged period of unusually low rainfall (Dalby and Church 1973) its effects have surely been increased by three large-scale but uncoordinated developmental aid projects. Throughout the whole of this vast area and beyond it, a huge international rinderpest vaccination scheme (begun in 1963) must have greatly reduced natural control over cattle fecundity. This effect was amplified by the large scale use of trypanocidal drugs. The ensuing onslaught on pastures, much reduced by drought, was made much more severe in some areas by the provision of boreholes. These, by giving ample uncontrolled water throughout the year, destroyed the system of transhumance whereby, for centuries, the cattle nomads rested their pastures. These operations, intended to ameliorate the harshness of life in the Sahel, had the opposite effect. Instead of saving life, they destroyed it.

How the recently promoted research on genetic and, now, biological control of the vectors of trypanosomiasis will succeed, one cannot foresee. Nevertheless it seems that techniques based upon these ideas, if properly applied, may have several advantages over anything so far devised for an attack on tsetse flies. Whatever the method used, however, certain problems must be solved — not to mention the proper assessment of the long-term consequences of its success upon the lives of local people. Unless the flybelt is isolated (as in Zululand or in several of the Nigerian *G. morsitans* belts) barriers against reinfestation must be created. Some projects concerned with temporary disease situations may not require artificial maintenance. Where an expanding population is exposed to infection, it may only be necessary to protect them during the period in which, by their own cultural activities, they transform a wildlife ecosystem to one sustained by cultivation and husbandry. However, these conditions will not arise frequently in the African tsetse zone.

Defence barriers may take the form of cleared, treeless corridors of sufficient width. These must be maintained either by cultivation (often not possible) or by repeated work to suppress regeneration of trees and shrubs. Supplementary works such as fences (to control animal movements) and "deflying chambers" (to cleanse road traffic leaving the infested area), may also be needed. An alternative is the periodic application of residual insecticides to potential tsetse resting places throughout a wide protective zone. To workers who have been involved in the formidable task of maintaining the efficiency of cleared barriers, the notion of an insecticide barrier is attractive. Here, however, there is a commitment to continual vehicle and machinery maintenance. This, as in the case of the insecticides themselves, depends ultimately upon a supply chain to European and American factories. If this chain is interrupted for only a few weeks, the whole control system may collapse. (Where, as in semi-arid environments, only the *palpalis*-group is involved, the defence problem is restricted to narrow lines of riverine vegetation. It therefore presents a less formidable problem than the continuous *morsitans* bush.)

For the people of Africa, a disadvantage of insecticidal techniques is dependence

upon the industrially developed countries for machinery and materials. A possible great advantage of biocontrol methods is that they must — if ultimately they are to succeed — become increasingly African-run and so independent of external sources of supply (see IDRC 1974). A biological control program, dependent upon the release of predators, parasites, and pathogens or an autocidal one calling for the release of sterilized males, offers other advantages. The possibility is presented of abandoning expensive and unproductive defence lines and substituting for them moving fronts sustained by the artificial build-up of biocontrol agents of African origin in locally run laboratories, and of having agents distributed, and their effectiveness measured, by indigenous staff. — J. Ford and S.N. Okiwelu.



Status and Future of Control

In spite of some recent advances in the field of its epidemiology and control, human and animal *Glossina*-borne African trypanosomiasis continues to present a formidable challenge to health and economy in many countries of tropical Africa by crippling animal husbandry and causing sleeping sickness. This fatal disease in man is periodically reported from many widely scattered areas, and has recently appeared in an epidemic form in several countries of the continent. It is estimated that the threat of trypanosomiasis persists today over at least 10 million km^2 of tropical Africa, including 7 million km^2 that would be suitable for the grazing of livestock, should the disease be fully controlled (FAO 1974).

Of the six main *Glossina*-borne *Trypanosoma* species of socioeconomic importance occurring in Africa, four affect livetock and have wild animal reservoirs (*T. brucei, T. congolense, T. simiae,* and *T. vivax*), one is a zoonosis with acute clinical forms in man and discrete infections in cattle and game (*T. rhodesiense*), and the last is mostly, if not entirely, restricted to man (*T. gambiense*) (MacLennan 1975). Drugs are available for individual and mass treatments and can be used to prevent infection. However, they have unpleasant, and sometimes dramatic side effects. Furthermore, there is increasing evidence of the development of strains of the parasites resistant to many of the compounds currently available, while no new drug has been developed in recent years by the chemical industry (Finelle 1975; MacLennan 1975). The development of immunization methods is under serious consideration but will probably require many years before reaching an operational stage. To totally eliminate the feral reservoirs, while simultaneously treating all the livestock with chemotherapeutics, constitutes neither a feasible nor even an acceptable proposal. At present, *Glossina* control thus constitutes the most promising approach to the enduring elimination of the disease. Ideally, and on a long-term basis, vector eradication would represent a cheaper and much more realistic solution than control campaigns, which must be carried out year after year without any time constraints and despite the high recurrent costs (MacLennan and Na'isa 1973).

Partly as a consequence of the catastrophic drought that recently affected the African continent (Temple and Thomas 1973), and partly because of the long-felt need to rapidly increase agricultural production, the FAO (during the World Food Conference organized in Rome in November 1974) suggested implementing a long-term program which, over a period of 40 years, would eradicate tsetse flies and bring trypanosomiasis under control over 7 million km^2 of potentially suitable grazing land (FAO 1974). The Conference endorsed this proposal. Such a program, after an extensive preparatory phase devoted to applied research, training, geographical reconnaissance, and socioeconomic studies, would involve the eradication of tsetse flies from about 200 000 km^2/year at an anticipated average annual cost of US$56 million, with the understanding that about 10% of the area might require treatments during several successive years to cope with both rein-

vasion by tsetse flies, and foreseeable operational weaknesses. Funding and implementation of the preparatory period is underway. The operational phase, which should begin around 1980, could in many ways follow the example of the policy earlier adopted in South Africa (du Toit et al. 1954) and in Nigeria more recently (MacLennan 1968; MacLennan and Na'isa 1973); but with the addition of all later technical, methodological, and operational improvements required by an endeavour of such magnitude.

Glossina Control Today

Since *Glossina* was first suspected of transmitting trypanosomiasis, many approaches were followed either to control tsetse or to prevent them from transmitting the disease. However, of these, the mass-release of parasites never gave good results, trapping had a very limited applicability except in a few special instances, bush-clearing and game extermination (quite apart from the fact that they are environmentally objectionable) usually had an unfavourable cost-effectiveness ratio, whereas genetic control methods were not found promising on a large scale. Therefore, for many years, insecticides constituted the only alternative. Their application, based on a sound knowledge of the target tsetse species' resting places and feeding habits, and on the use of suitable equipment, has become cheaper with time (Finelle 1974; MacLennan 1968).

Dieldrin and DDT, and in some instances HCH, were the only insecticides used on a large scale and for extended periods, the first two mostly as residual sprays, the last mainly for fogging. Although the first large-scale tsetse eradication campaign had been carried out in South Africa with aircraft and many studies had been made of the aerial application of insecticides for *Glossina* control, almost all the important control campaigns in the past were carried out with residual insecticides applied from the ground — either with hand operated sprayers or with vehicle-carried sprayers and mistblowers. If the residual effect lasts longer than the *Glossina* puparial period, one application can: (1) theoretically eradicate the vector from the treated area; and (2) usually achieve tsetse eradication. Full particulars on the methodologies used can be found in many recent reviews on the subject (Atkinson 1971; Baldry 1963, 1964b; Burnett 1970; Davies 1971; Ford 1971; Jordan 1974b; LeRoux 1974; Lycklama and Nijeholt 1968a, b; Riordan 1966; Touré et al. 1975).

On a long-term basis, however, the situation is not so simple. Because of the logistic difficulties involved, the ground application of insecticides to the vegetation of sparsely populated, and/or uninhabited areas is a time-consuming operation requiring much manpower and very thorough supervision (LeRoux 1974). As tsetse flies can fly relatively fast and far, move with game and cattle, and be dispersed over long distances by vehicles, reinvasion of the treated areas constitutes a permanent threat (Davies 1975). The costs of maintaining an effective barrier zone are often very high. In one of the best organized tsetse eradication programs, the elimination of the fly is being carried out at the rate of about 12 500 km^2/year, in the dry or moderately humid savannah zone of West Africa. This, in addition to the overall geographical reconnaissance, planning supervision, and evaluation activities, requires a small army of about 2000 people and 60 vehicles (Na'isa 1974). As well, about 10% of the area requires a second treatment for various reasons. Some parts must be treated even three or more times before eradication is achieved. In more humid zones difficulties are greater because: (1) the vegetation cover is denser and represents a greater proportion of the total area (Davies 1975); and (2) there are usually two wet seasons a year — with the result that only a brief period at the beginning of the longer dry season is fully convenient for the application of residual

The filling of back-pack sprayers for selective residual applications of DDT, Kalamaloué Reserve, Chari River Valley, Chad, (1 May 1973, M. Laird).

treatments. Therefore, late applications could result in the insecticide deposits being washed away by the rains before the last tsetse emerge from their underground puparia. Furthermore, the growth of the vegetation is faster than in the drier zones, and a large proportion of untreated resting places is rapidly provided within a few weeks of the application.

The need to cover large areas during the most appropriate period of the year reawakened interest in the aerial application of insecticides. Two different lines of approach were considered: (1) several sequential applications of nonpersistent insecticide deposits; and (2) a single application of a residual deposit.

Risks resulting from environmental contamination have always been kept in mind when planning and implementing large-scale pesticide applications for tsetse fly control (Graham 1964; Koeman and Hadden 1968; Langridge and Mugutu 1968); whereas obvious economic considerations have led to the use of the lowest effective dosages. However, the relative importance of environmental considerations has steadily increased to the point where they could

prevent the production of the residual insecticides presently employed for *Glossina* control (Djerassi et al. 1974; Jukes 1974), or interfere with the funding of large-scale and long-term programs based on their use. This has induced both a more thorough assessment of the impact of the tsetse control treatments on nontarget organisms (Koeman et al. 1971, 1974), and an acceleration of the search for economically and environmentally acceptable alternative insecticides and nonchemical control methods. The most promising alternative to date is based on the mass release of sterile males (Itard 1975a), whereas the use of parasites, pathogens, and predators is also under study (Gruvel 1974b).

The Search for and Evaluation of Insecticides

The first systematic investigations carried out for screening new insecticides that could be used for *Glossina* control began about 15 years ago. These investigations were oriented toward the development of compounds and formulations that could be applied from aircraft (Burnett 1961, 1963). Some of the more promising compounds were subsequently used, either for experimental purposes (Burnett 1962; Burnett and Thompson 1956; Burnett et al. 1961, 1964; Foster et al. 1961; Hocking and Yeo 1953; Hocking et al. 1953a, b, 1954a, b, c, 1966; Irving and Beesley 1969; Irving et al. 1968; Lee 1969; Tarimo 1971a; Tarimo et al. 1970, 1971a, b, 1972; Thompson 1953) or for tsetse control operations. One of them, endosulfan, is now widely used in East Africa (Anonymous 1973; Kendrick and Alsop 1974; Park et al. 1972; Robertson 1971).

After several years of interruption, these investigations were resumed as part of the global WHO Programme for Evaluating and Testing New Insecticides (WHO 1971, 1974). Both the residual contact activity and intrinsic toxicity of the candidate chemicals were used as a basis for the selection of new insecticides for tsetse control that were to be tried as residual deposits as well as ultra-low-volume (ULV) applications (Hadaway 1972; Hadaway and Turner 1975; Riordan 1971). Some of the most promising compounds have been experimentally used under operational conditions, but from a cost-effectiveness viewpoint, the results obtained to date have not been very encouraging (Challier and Lorand 1972; Challier et al. 1974; Spielberger and Na'isa 1975; Tarimo et al. 1973).

Investigations are also under way to assess the potential of the new chemicals to accumulate through food chains and to persist in the environment. Some systematic studies have been undertaken to determine the impact of dieldrin upon nontarget organisms, when it is applied to riverine vegetation as a residual deposit against tsetse, either from the ground or from the air (Koeman et al. 1971, 1974; Touré et al. 1975).

The Aerial Application of Insecticides for *Glossina* Control

Low- and Ultra-Low-Volume Applications

The aerial application of insecticides at low- and ultra-low-volume, and therefore dosage, for *Glossina* control does not produce persistent deposits on the vegetation of the treated area (Lofgren 1970). The purpose of this treatment is therefore restricted to the complete or almost complete destruction of the population of adult flies existing within the zone under attack at the time of treatment. To ensure lasting results, sequential applications must be carried out so that the young female flies that have emerged from puparia since the previous treatment are killed before depositing their first larva. Population dynamics and simulation studies have indicated that it could be less expensive to aim, not for the complete elimination of tsetse flies on the wing during any given

A small Cessna aircraft fitted with a wind driven rotary atomizer for producing an insecticide aerosol. Large areas are treated with small quantities of insecticide to control savannah species of tsetse (WHO photo, J.D. Parker).

treatment, but rather, for an increase in the number of treatments; on the assumption that below a certain density, isolated flies do not find mates and die without producing progeny. Unfortunately, tsetse have an incredible ability to survive and to reproduce at extremely low densities, as observed during many eradication programs. It is thus safer to use an effective dosage, and to carry out a number of applications allowing for a reasonable margin of safety. In the highlands of East Africa, the most commonly used schedule involves five applications 3 weeks apart.

To be fully effective, ULV applications must be made in such a way that almost all the droplets have an insecticide content sufficient to kill a single tsetse, while lacking an excess of toxicant. This implies that a narrow droplet-size spectrum should be used.

It is also very important that the number of droplets per unit of surface area or volume ensures a fair chance that each tsetse is hit by at least one droplet. This means that the droplets should be both numerous, and small enough to float in the air for long enough to drift over an area much larger than the aircraft wingspan before settling on the ground or on vegetation or being lost (through evaporation, by drifting away from the target area, etc.). These conditions can only be met by a few insecticides that: (1) have a high intrinsic toxicity for tsetse; (2) are liquid in their technical form at room temperature; and (3) can be formulated as liquids without excessive dilution. Experience has established that the most appropriate droplet size is about 20–30 μm (VMD). In view of the above, this demands compounds having a medium lethal dose for a tsetse fly of about 1–12 ng/fly. Among the insecticides fulfilling these conditions are some synthetic

Insecticide applications being made to tsetse habitat in Chad with a light helicopter. A thermal fogging technique is being used to make an aerosol application (WHO photo).

pyrethroids, fenthion, and some newer organophosphorus compounds, e.g. dieldrin and endosulfan (Hadaway and Turner 1975). Endosulfan is extensively used for the purpose because it is relatively cheap and does not persist in the environment. However, it is acutely toxic to fish.

The fate of small droplets applied from a low-flying aircraft depends to a large extent upon air currents, as significant upward air movement will disperse them through the atmosphere. Therefore, in practice, only a few hours per day (usually in the early morning and late evening) are convenient for such applications. Night operations are carried out (Kendrick and Alsop 1974; Lee et al. 1975), but they raise other serious operational problems. It could be more difficult to hit resting flies at night than active flies that are very often on the wing.

Nonresidual aerial applications are made using either a thermal fogging device (Hocking and Yeo 1953; Park et al. 1972), in which an insecticide formulation is injected into the exhaust pipe of the aircraft, or with some form of rotary atomizer (Irving and Beesley 1969; Irving et al. 1968; Kendrick and Alsop 1974).

Using endosulfan, the first large-scale

ULV applications were carried out with a very large safety factor. Dosages of about 30 g/ha for each application were employed. With improvements in equipment design (Lee et al. 1969) and insecticide formulation, *Glossina* control campaigns have recently been carried out with dosages as low as 6 g/ha (Kendrick and Alsop 1974; Lee et al. 1975). The use of pyrethrum (Irving et al. 1968; Tarimo et al. 1970, 1971a, b, 1972) has given inconsistent results. More recently, a synthetic pyrethroid was used on an experimental basis (Lee et al. 1975), but the dosage was too low (0.6–1.2 g/ha) and the results were inconclusive.

Aerial ULV applications of insecticides have been rather successful in the wooded savannah areas of East Africa (Anonymous 1973; Kendrick and Alsop 1974; Park et al. 1972; Robertson 1971), and could probably be adjusted for increased flexibility and effectiveness (Bals 1973; Barlow and Hadaway 1974; Maas 1971; Niessen 1974; Tarimo 1971b). It would be desirable to assess the value and limitations of the method in the savannah zones of West Africa, especially in areas where the wooded savannah is intermingled with gallery forests that constitute the main resting sites of the tsetse flies during most of the dry season. It was recently shown that only a small proportion of the insecticide applied over such a gallery forest reaches ground level where the tsetse are both active and at rest during daylight hours (Johnstone et al. 1974).

High Volume Applications

The aerial application of insecticide at high volume, and usually high dosage, enables a long-lasting residue to be deposited upon the vegetation. Under ideal conditions, a single application is sufficient to eliminate tsetse from the treated area. The purpose of the treatment is thus the same as that of residual applications made from the ground. The inherent advantages of aerial application are that: (1) it does not require an elaborate infrastructure and a large number of staff and vehicles; and (2) large areas can be treated in a relatively short period of time. One obvious disadvantage is that most of the insecticide droplets settle on the upper leaves and branches (sites not considered as favoured resting places for the fly).

This technique was used experimentally — with a great degree of success — against *G. pallidipes* inhabiting dense thickets in the Lambwe Valley, Kenya; dieldrin being applied as an invert emulsion requiring special application equipment. After the initial demonstration of the feasibility of such an approach, the trials were abandoned (Allsop and Baldry 1972; Baldry 1971; LeRoux and Platt 1968). Some investigations were also undertaken in East Africa using aircraft applications of defoliants at a high dosage as a preparation for the subsequent application of insecticides (Tarimo et al. 1974).

In West Africa, helicopter application of insecticides at high volume has been used, both routinely and for experimental purposes (Bauer 1971), against both riverine tsetse flies and *G. m. submorsitans* (which at the height of the dry season behaves almost like a riverine species). Outstanding successes were achieved in Niger and Nigeria with dieldrin (1000–2000 g/ha), endosulfan (1500 g/ha) and a mixture of DDT, HCH, and dieldrin (respectively 1150, 450, and 450 g/ha) (Spielberger 1971; Spielberger and Abdurrahim 1971, 1975; Spielberger and Na'isa 1975; Spielberger and von Sivers 1970; Spielberger et al. 1971). Less satisfactory results were obtained with other insecticides that had been tentatively selected in view of their greater environmental safety. In the case of the investigations carried out in Mali, this lack of success might have been due to operational and equipment problems (Challier et al. 1974; Spielberger and Na'isa 1975).

At high volume, and especially when the target is not an open or flat wooded savannah but a gallery forest and its immediate surroundings, the occurrence of extensive insecticide drift constitutes an obvious disadvantage. This is avoided by

Piper Pawnee spraying dieldrin, Otuok/Riamkanga Area, Lambwe Valley, Kenya (D. Baldry).

using larger droplets of about 65–150 μm (VMD). As already indicated, droplets of that size settle mainly in the upper canopy and only 1–1.5% of the insecticide reaches ground level within the gallery forest (Johnstone et al. 1974). Nevertheless, field trials have shown that this can be sufficient to kill tsetse, perhaps because, at certain periods of the day, *Glossina* rest on the ground vegetation, close to, but outside of, the gallery forest. It is also strongly suspected that at night the gallery forest canopy constitutes an important tsetse resting site (MacLennan and Na'isa 1973). These aspects of the riverine and riverine-like *Glossina* have so far been inadequately investigated (Glover 1967). Further studies might allow a more judicious use of insecticide. Investigations are in progress on the insecticide formulations used for such purposes. Their results may lead to a reduction in the dosage of active ingredient by the formation of insecticide deposits combining longer persistence with better availability to the tsetse (Barlow and Hadaway 1974; Niessen 1974).

The immediate impact of these high-volume dieldrin applications on nontarget organisms in the gallery forests can be dramatic (Koeman et al. 1974), but the long-term effects have not yet been assessed. As long as the actual treatments involve a small fraction of a small area (for example, the gallery forest and its immediate surroundings), there is a likelihood of repopulation of the treated zone by birds, mammals, and insects from the immediate vicinity of the treated zone (Langridge and Mugutu 1968). The situation could be less favourable if the treatments covered tens of thousands of square kilometres over a short period of time. It must be further noted that during the dry season, such gallery forests usually shelter most of the

wildlife and other nontarget organisms of the savannah zones of West Africa. The elimination of the species resting there during that period could be as complete and durable as that of tsetse flies. Such a risk must, therefore, be most thoughtfully assessed before the authorization of high-volume applications from aircraft over very large areas (FAO 1974).

Insecticide Applications and Integrated Control Approaches

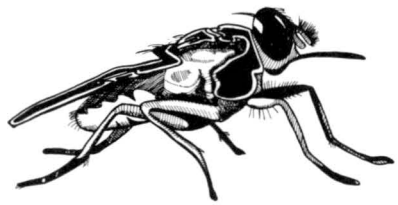
Although the use of pheromones and other attractants associated with traps cannot be discounted, it seems most probable that in the near future integrated *Glossina* control methods will often be based on the combination of insecticide applications and the release of sterile male flies (Itard 1975a). In the more distant future, parasites, predators, and pathogens might also be used to complement the action of insecticides (Gruvel 1975a).

Residual applications of insecticides, whether from the ground or from the air, would interfere for a lengthy period with the inundative release of sterile males, predators, and parasitoids, but not with the use of pathogens. The interval between the last insecticide application and the beginning of the sterile-male fly (or predators, or parasites) release, should therefore be adjusted to the effective residual life of the insecticide deposit, as well as to the population dynamics of the tsetse species under attack. Under the most favourable conditions the releases could probably be safely made the year following the application of residual deposits. Under less favourable conditions, it would be unsafe to wait more than a few months (Anonymous 1972).

Sequential ULV applications of insecticides, made from the air or even from the ground (Challier et al. 1964; Kernaghan 1954; Tarimo 1974), have little residual effect and therefore constitute the best approach when preparing for the subsequent use of any biological or genetic control method.

It might appear simpler to carry out additional ULV applications or another round of residual spraying — or to increase the dosage of the insecticide used — than to follow up the insecticide treatments by using nonchemical control methods. However, it is always the last couple of tsetse flies that are the most costly to kill, while the application of chemicals always interferes to some extent with the equilibrium of the treated ecosystems. From a purely theoretical cost-effectiveness viewpoint, integrated control measures may therefore be as efficient as, and cheaper than, chemical control methods used alone (WHO 1975). One of the potential advantages of integrated control measures is that sterile males, parasites, predators, and pathogens may reach small residual *Glossina* populations surviving insecticide applications because they were living in unsuspected microniches that were not treated. These individuals, just because they are atypical, cannot be easily eliminated by increasing the insecticide dosage and/or number of applications (Touré et al. 1975). These hypotheses have yet to be tested through actual operations.

In a number of instances, *Glossina* eradication, even if constituting the long-term objective, may not be economically feasible or justified by socioeconomic studies; whereas, a considerable reduction of tsetse fly abundance is required to curb sleeping sickness epidemics, to settle people, or to establish trypanosomiasis-tolerant cattle (Ford 1969). Under such conditions the sequential use of insecticides and nonchemical agents might constitute a suitable long-term solution. For these purposes the release of other biological agents would be preferable to the release of sterile males, because the vectorial capacity of male tsetse is not impaired by sterilization, unless their lifespan is considerably shortened. — J. Hamon, D.A.T. Baldry, J.D. Parker, A. Challier, and A.R. Stiles.



Predators

During the course of their field work, many specialists on tsetse flies have observed the predatory action of various vertebrates and invertebrates on *Glossina*. Observations of these predators are neither very numerous, nor adequately specific. Furthermore, the records are scattered and frequently anecdotal. Totally absorbed by the study of tsetse flies, research workers have tended to neglect or give little attention to the fauna associated with the flies (except as regards the vertebrates on which they are likely to feed).

At present, the fight against disease-carrying insects, which attack humans or animals, is being directed more and more toward the use of natural enemies of the target vector. Thus, biological control has become of great interest to scientists. Its application, however, requires extensive preliminary work based on sound knowledge of the various elements to be utilized. Concerning the role that may be played by predators in the fight against *Glossina*, it is regrettable to have to concede that those really meriting attention are rare, and that studies concerning them are still very incomplete.

It is therefore pertinent to assess our knowledge of the subject by: (1) reviewing the cases of predation mentioned by various authors; and (2) examining the relationships of the predators with each of the development stages of tsetse flies in relation to their ecology. Some fundamental principles might be drawn from these data, which could lead to the application of predation, in parallel with various parasites and/or pathogens, in vector control programs.

A predator may be defined as a free ranging organism that searches for, and kills, live prey as food. However, one is tempted to enlarge this definition in the light of problems associated with the biological control of *Glossina*, and to consider as a predator any living organism capable of removing from the environment a certain number of individuals without necessarily eating them. The significance of this enlargement of the definition will become obvious later.

The Predators of Tsetse

During more than a century of observations, numerous instances of predation upon tsetse flies have been reported (Buxton 1955; Gruvel 1974b, 1975a). The predators belong to widely different groups.

Vertebrates

Mammals

It has often been asserted that monkeys and baboons (*Papio* spp.) are so quick in their reactions that not only are *Glossina* unable to feed on them but also, the latter can catch tsetse!

Bats moving in the semi-darkness of gallery forests have yet to be confirmed as predators of *Glossina*. However, there is no evidence that tsetse flies are not, at one time or another, part of their diet (Gruvel 1974a).

Swynnerton (1936) stated that the mongoose may destroy an important quantity of *G. morsitans* puparia in Tanzania. He also observed traces of scraping in larviposition sites of *G. austeni* accompanied by

footprints and excreta of the East African elephant shrew (*Petrodrimys tetradactylus*), which he suspected of eating puparia and other shallowly buried insects.

Lloyd (1914) recorded observations suggesting that adult *G. morsitans* provoke real excitement in shrews, small rodents, and mongoose when introduced into the cages of these animals.

Birds

Guinea fowl and francolins are often found in the same biotopes as *Glossina*. While scratching, they investigate almost the entire ground surface of the area, and could not fail to uncover and swallow a significant number of puparia. Nevertheless, the remains of such meals are rather rare in the crops of these birds. Simpson (1918) found no trace of *Glossina* in an examination of the contents of 379 stomachs.

On the other hand, Swynnerton (1936) reported having observed three species of birds (*Dicrurus* sp. and *Bradornis* spp.) feeding on tsetse flies. He also observed other birds feeding on glossinids that were resting on the small branches or the bark of trees.

A few authors (see Swynnerton 1936) have mentioned the presence of tsetse fragments in bird crops, particularly of granivores that scratch the ground, and insectivores.

Reptiles

Little is known of the influence of reptiles on tsetse flies. In his dissections of lizards, Simpson (1918) was unable to identify remains of these insects. However, predation by lizards in a large experimental cage placed in a *G. tachinoides* site was the only plausible explanation for the disappearance of a large number of flies introduced into the cage, which was free of any construction defect that could have allowed the insects to escape (Gruvel 1970a).

Invertebrates

Invertebrates (specifically, various insects and spiders) have a more obvious role as predators of *Glossina* than do vertebrates.

Insects

Odonata, Coleoptera, and Orthoptera play a role, but to a less uniform degree than Hymenoptera or asilid Diptera.

Odonata

Dragonflies attack tsetse flies, or at least appear to do so. Carpenter (1913) identified an abundant dragonfly *Cacergates* sp. as an enemy of *G. palpalis*. Lamborn (1915b) frequently observed *Orthetrum chrysostigma* catching *G. morsitans* on men's backs. Campion (1921) recorded both *O. chrysostigma* and *O. brachiale* as preying upon *G. morsitans*.

Coleoptera

After studying *G. palpalis* in Uganda, Fiske (1920) concluded that certain adult and larval Coleoptera of the families Carabidae and Elateridae may destroy large quantities of puparia. Their effect appeared to him particularly important at unusual larviposition sites. Of a total of 9000 puparia, he estimated the mean loss at 7%, with variations of 0–31% depending on the locality. In Tanzania, Nash (1933a, b) observed *Melyris pallidiventris* larvae (Coleoptera, Melyridae) devouring tsetse puparia. Much earlier, there had been reference to tiger-beetles (Cicindelidae) preying upon tsetse — the first of them due to David Livingstone in the 1850s (Laird 1975a).

Orthoptera

Challier (1971a, b), while studying *G. p. gambiensis*, noted the presence of large crickets on the larviposition sites and considered them to be occasional predators of puparia.

Hymenoptera

Several investigators have reported hymenopteran predation on tsetse flies. Simpson (1918) described *Bombex* as the most voracious enemy of *G. morsitans*. This was confirmed by Nash (1933a, b) and by Fiske (1920) for *G. palpalis*. On the other hand, in what is now Zaïre, Bouvier (1936) frequently noted the presence of *G. palpalis* in nests of *Sphex, Synagris*, and *Bombex*.

Ants are often mentioned as enemies of tsetse larvae or puparia. For example, members of the genera *Euponera* (*E. senaarensis*) and *Paltothyreus* (*P. tarsatus*) have been observed carrying away *G. morsitans* and *G. palpalis* larvae (Carpenter 1912). In contrast, Lamborn (1916) and Simpson (1918) both stated that newly deposited larvae were not attacked by ants. They attributed this to the presence of a glossinid secretion that repels these predators. In Tanzania, Ford (1940) studied in detail the predation of ants upon *G. swynnertoni* puparia. He observed members of the genus *Pheidole* carrying puparia into their nests, and considered these ants to be very efficient predators. One of the first to attempt a quantitative estimation of the inroads of these predators into tsetse populations, he compared the decrease in numbers of puparia of *G. swynnertoni* artificially distributed, according to a definite plan, in: (1) a normal forest habitat; and (2) a savannah habitat, which had not, for a long time, suffered bush fires. Ford's (1940) estimated predation rates on puparia were 11–18% in the first case, and 25–44% in the second. These experiments, repeated by Whiteside and Kemp (cited by Potts 1950) showed that the intensity of predation is related to the coexistence of two distinct types of vegetation at the same time of year, and that it occurs when the choice of prey becomes smaller for the ants.

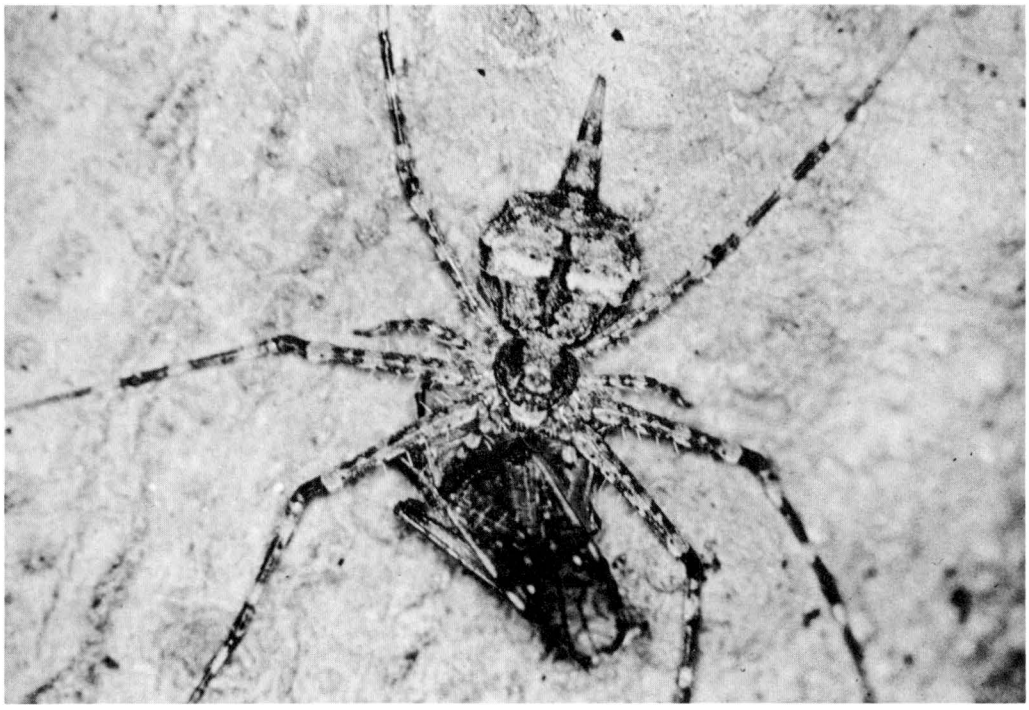
In Uganda, Rogers (personal communication) studied predation on laboratory-reared puparia that had been transferred to a natural site and placed at varying distances from one another. He concluded that the predation was due to members of the genus *Pheidole*. The results were analyzed statistically, to determine the degree to which the ants' discovery of puparia was random. At high puparial density, once the predators had encountered the first puparium, they proceeded to locate all others in the vicinity. On the other hand, when the spacing between puparia increased, discovery became random. Rogers' studies are an example of both the use of, and the importance of, mathematical methods in interpreting the action of predators (and for that matter, of parasitoids).

Diptera

Like ants, asilid Diptera are well-known predators of adult tsetse, as well as other insects active in the same biotopes.

Generally, asilids hunt in a wide variety of habitats and are actively oriented toward insects passing within their reach. They lie in ambush at various heights, characteristically at the tips of small isolated branches from where they keep watch over their surroundings. They are, at this time, totally immobile. Outwardly indifferent to what is happening around them, they are nevertheless poised to attack any insect crossing their field of vision. They quickly attack and then, after a brief flight, return to the ambush site either to resume their watch or devour the prey they have captured. These sorties are repeated many times during the day.

The asilids most likely to capture tsetse flies of the forest galleries are those that hunt both in the galleries themselves and in their immediate vicinity. Their ambuscades depend on the species, with the location and height from the ground varying with the chosen plants. These range from tall grasses outside the galleries to small open branches inside. In studies in the forest galleries of the lower Chari River, tsetse flies (*G. tachinoides*) were not observed to be captured by asilids living in the same habitat although, at certain periods of the year, the former were the only flying insects. These asilids were most of-



Hersiliid spider with captured tsetse adult, on tree bark, Chad (J. Gruvel).

ten observed to prey on Hemiptera, small Orthoptera, or Coleoptera (Gruvel 1974a, b). However, under identical ecological conditions in Nigeria, a few observations were made of examples of the capture of *Glossina*. Southon (1959), in a study conducted in eastern Africa, suggested that *G. swynnertoni* might constitute 15% of the prey of 330 asilids caught while they were feeding. The rapidity with which tsetse can be captured and eaten may partly explain the scarcity of relevant observations.

Spiders

Spiders are highly ranked predators of adult *Glossina*. There are numerous reports of frequent captures by some species that are particularly well equipped to seize tsetse flies. Indeed, the detailed study of the predatory action of one of these species led to the first real estimation of the effect of predation on the decline of tsetse populations.

Spiders of the genus *Nephilia* are common in central and eastern Africa where they weave their immense webs between trees. These webs are strong enough to snare, not only insects, but also small birds (observations by Fiske (1920) in the region of Lake Victoria). Moreover, secondary webs woven by other spiders and attached to the larger one, form a complex network that acts as a filter, which tsetse find difficult to avoid. Fiske referred to the capture of *G. palpalis* in such quantities as to contribute to the reduction of their population. Chorley (1958) and Glasgow (1963) confirmed the importance of this predatory activity of *Nephilia*, stressing the role that they play in controlling prey populations. On the other hand, Harris (1930) noted that in Zululand *G. pallidipes* were rarely captured by these spider webs, even though nephiliids were abundant.

Jumping spiders of the family Attidae (*Plexippus paykulli*) catch large numbers of *G. palpalis* in Gambia, while ignoring

other flies (Simpson 1918). Swynnerton (1936) confirmed the fact that resting tsetse may be killed by jumping spiders.

Hersilia setifrons Lawrence (family Hersiliidae) is a spider that lives on the trunks and larger branches of various trees. It pounces upon resting tsetse adults, which it enmeshes and immobilizes within a silken envelope. Because it does not eat the prey at once, it is relatively easy to study this instance of predation by counting the number of *H. setifrons'* silken "packages" on tree trunks.

This sign enabled Southon (1959), helped by a dozen observers, to make a quantitative assessment of the role of these spiders in reducing *G. swynnertoni* populations. From 100 to 350 insects imprisoned by *H. setifrons* were located monthly during 7 months of observations. It was found by dissection that *G. swynnertoni* constituted up to 3% of the prey. Southon (1959) estimated that at 1400 spiders/ha (the minimum population of *H. setifrons*), with tsetse averaging 75/ha and bearing in mind that each spider feeds once weekly, the predation rate each week was 17%. However, only 1% of the prey consists of tsetse flies.

Spiders of the genus *Hersilia* are found during all seasons in the forest galleries of the lower Chari River, the characteristic plants of this *G. tachinoides* habitat being *Morelia senegalensis* and *Mitragyna inermis* (Gruvel 1974a). These hersiliids reach their maximum abundance during the hot season. Their coloration makes them almost indistinguishable from the bark of the trees that they haunt, and their behaviour is as described above. It should be noted that at the moment of capture, the attention of a nearby observer may be attracted by a sharp buzzing produced by the wings of the *Glossina*. It is interesting to note that the fly is not paralyzed by the spider and that it can resume flying if released from the enclosing web. The predatory role of this spider on resting *G. tachinoides* can be observed at any time of the day — or sometimes even at night, at full moon. Captures are particularly numerous during the hot season, being made easier due to the torpor that then overcomes tsetse flies.

The quantitative importance of these captures was not assessed. However, in the locality where these observations were made, the density of *Hersilia* is highest early in March. It is maintained at a high level through April and May. During this period, the tsetse flies congregate in groups on the tree trunks, resting for several hours at the height of the day. One day in April, 22 spiders were counted on a single *Morelia* trunk. On the same tree, 10 captures were witnessed within 2 h. The high concentration of spiders on *Morelia* trunks during the hot season, which corresponds to the period of congregation of resting *G. tachinoides*, ensures efficient predation during the three hottest months of the year, and allows *Hersilia* to exert a decided limiting effect on the tsetse population (although the author has never recorded a predation rate as high as that published by Southon 1959). *Glossina* is not the only prey of these spiders, as indicated above. Despite the comparative scarcity of *Hersilia* during the cool season, these spiders then catch other insects which haunt tree trunks, notably pyrrhocorid Hemiptera.

Predator-Tsetse Relationships

Predators on *Glossina* are varied. Some among them, notably vertebrates, play a still-unknown role; others, including other insects and spiders, often exert a decided effect on adult and immature tsetse populations.

Many uncertainties and unknowns are obvious as regards both the predators themselves, and predator-prey relationships. These deficiencies are due to the inadequacy of research on the relationships between the tsetse flies and the fauna sharing their biotopes, and to the field observer's difficulty in witnessing the actual moment of contact between *Glossina* adults and their natural enemies.

There is no evidence to show any spec-

ificity of action of any of the predators upon tsetse. The latters' capture is therefore the result of a chance encounter, determined by ecological conditions.

Predators

Very little is known of the biology of the predators of *Glossina*, even that of arthropods, whose action against *Glossina* is indisputable and relatively common. The known aspects of their behaviour have already been mentioned. Their ecology has not been adequately studied: the variations of their habitats; their longevity; their breeding periods; the size of their populations; and the frequency of their meals remain practically unknown. Information on these points would enable one to judge their real importance and their efficiency in contributing to the natural limitation of tsetse populations.

One can only theorize that predation is facilitated by the great mobility shown by some predators when seeking their prey, and is more efficient when the predators are abundant and concentrated in the territories occupied by adult *Glossina*.

Tsetse Flies

In contrast, tsetse-fly ecology is well understood (or rather, the ecology of the species more commonly dispersed in the savannah regions is well understood, but unfortunately there is still a lack of knowledge about tsetse flies that are strict dwellers of the great equatorial forest).

In the light of information gathered during ecological studies, it is possible to pinpoint those periods in their life cycle when the *Glossina* are particularly vulnerable. They may of course be attacked at all stages of their development: larval, puparial, or adult.

Larvae

In nature, larvae are deposited on the ground in places selected by the tsetse females. Assisted by their tropisms, larvae complete the next phase of this act of selection, coming to rest in locations where the future puparia will find the most favourable conditions for their development. Larviposition sites, usually located in the normal habitats of the adults, have the following general characteristics: (1) protection from intense sunlight by more or less high foliage, low plants, fallen trees, and natural cavities in the ground or in trees; and (2) loose soil at the surface, often trampled by animals and covered with leaves, overlaying a more humid substrate.

Normally the free-living larval stage is of very short duration. A few minutes after being deposited, the larva buries itself shallowly after little or no movement over the surface of the ground. The factors affecting the behaviour of the larva and consequently the duration of the larval stage are soil texture, light, and humidity. A positive geotropism and a negative phototropism ensure the burrowing reflex, which is facilitated by a loose soil. Penetration is that much more immediate where soil humidity is high (without, however, having reached or even being near the saturation point). Larvae are baulked by a substrate that is either too dry or too humid. Puparia thus develop below the surface at the interface of dry and humid layers, which they cannot penetrate because of the compactness.

If the larva encounters adverse conditions (soil too hard, too dry, or too wet), its life is appreciably prolonged. It then wanders over the surface for some time (up to or exceeding 1 h), until it finally stops and pupates; usually without burying itself. Puparia so formed evidently have a rather uncertain future.

Because of its usual burrowing speed, the larva is almost totally protected from possible predators. It is only when conditions are adverse, when they wander as described above, that larvae become more vulnerable. These particular conditions occur periodically in the forest galleries of the savannah regions during the onset of

the dry and hot seasons. Various soil-digging predators, such as ants, may then catch some. I have never personally observed this type of predation in the various *G. tachinoides* habitats in Chad and Cameroon. Observations by Lamborn (1915a, b; 1916) and a few others seem to apply to a provoked contact, not to a natural one.

Puparia

These are located in places commonly designated as larviposition sites, having the general characteristics outlined above. The sites depend on the nature of the soil which, in addition to appropriate physicochemical conditions, ensures the persistence of a protecting plant cover. Generally, the immediate environment of the puparia is relatively uniform due to the adaptive movements that make pupation possible at all seasons except — though rarely — where general climatic conditions are adverse.

Humidity at the puparium level varies, but is always between 48 and 84% (Atkinson 1971). This hygrometric range was confirmed for larviposition sites of *G. tachinoides*. However, optimum hatching rates were recorded when puparia formed at sites where the soil humidity was above 60% and in the vicinity of 80% (Gruvel 1974b).

The duration of pupation is inversely proportional to the temperature to which the puparia are exposed. In the lower Chari River Valley where a short rainy season is followed by a long dry season (the latter being characterized by a cool and a hot period), temperatures at the level of the puparia range, on average, from 22 to 28 °C between these two periods. This results in a pupation period 1.6 times longer during the cool season (av. 38 days) than the hot one (av. 23 days). These variations are naturally much smaller in regions where the climate is characterized by a narrow range of temperatures. The lengthening of the pupation period increases the interval during which puparia are exposed to their natural enemies.

In regions where the general climate and rainfall show large annual variations, there is periodical concentration and dispersal of larviposition sites. Although it is practically impossible to estimate puparial losses, these are certainly higher during periods of concentration, which coincide with the species' most extreme climatic conditions. These conditions, by causing concentration of the local fauna, increase the chances of contact between predators and prey. Insectivorous vertebrates scratching the soil, Coleoptera, Orthoptera, Hymenoptera/Formicidae may then use tsetse puparia as their main food source.

Adults

At Hatching

On emergence from the ground, tsetse flies spend a long time stretching their wings, legs, abdomen, and proboscis. They are able to fly only after a delay varying from 1 to as much as 2 h. During this time, they can walk and are sometimes active. In the case of *G. swynnertoni*, Southon (1959) noted that flies at this stage remained where they were hatched for nearly 2 h, finally flying off directly from this point. The only displacements appeared to be caused by the scuffle resulting from other flies hatching from neighbouring puparia, or from encounters with other terrestrial insects such as beetles or ants. When thus disturbed, the flies move away a few centimetres and become immobile again.

This waiting phase prior to the first flight makes the young adults vulnerable to carnivorous insects or any other insectivore.

At Rest

Resting tsetse may be found on the trunks or branches of trees and bushes comprising or bordering their habitats. They occupy different sites on the vegetation, being found at different heights by day and by night. The altitudes at which tsetse rest are a characteristic element of their ecology.

Many observers have noted variations in the height of the diurnal resting places according to time of day or season, suspecting that climatic conditions have an influence on these variations. An assessment of the major microclimatic factors at the resting sites shows that tsetse flies prefer humid sites that afford conditions of lower light intensity and temperature than the environment where the flies are normally active. Temperature appears to be the most important factor in determining the altitude of the resting places. For *G. tachinoides* in the lower Chari River Valley, Gruvel (1974a) demonstrated a relationship between environmental temperature and the mean height of resting tsetse. The average height of the resting places decreased as the ambient temperature increased in the range 31–41 °C. At temperatures below 15 °C resting flies are more scattered; whereas, at high temperatures they are closely congregated, and are always found at heights below 30 cm. Regardless of the temperature of the environment, the temperature of the resting places of adult *G. tachinoides* never exceeds 33 °C.

The remarkable assemblage of resting glossinids at the base of tree trunks, during the hottest periods of the year, accounts for the large number of observations of captures by various predators, which also live on these trunks. Although this must be proven, the predators are probably reacting similarly to climatic (especially thermal) variations and therefore also become more concentrated. Resting tsetse are thus exposed to more attacks by the habitual fauna of tree-trunks. Depending on the region, this includes insect predators of the families Mantidae, Reduviidae, and Tettigoniidae, spiders of the families Attidae and (especially) Hersiliidae, and vertebrates such as lizards.

At night, tsetse flies rest on leaves in the vicinity of the flies' normal daytime habitats. One can only theorize on nocturnal predation. However, under a full moon I have observed the capture of a *G. tachinoides* by a hersiliid spider.[2]

One may ask, with Rogers (personal communication), if the resting places are not also chosen in the course of a 24-h period in the interests of escaping diurnal and nocturnal predators. Resting places change at dusk. At night, tsetse flies resting on leaves may detect the approach of invertebrate predators by vibrations transmitted through their legs. During the day, when the flies are resting on tree trunks, they do not perceive such vibrations. Then, however, they more easily escape predators hunting by sight, such as birds.

In Activity

The activities of adult *Glossina* spp. are governed by hunger in the case of both sexes or by the search for females by males. These activities consist of movements that, depending on climatic conditions, lead the flies to disperse from their original habitat, which they either eventually return to, or abandon for other biotopes. Tsetse are thus active over quite a considerable area.

Daily activity rhythms differ from one season to the next. In regions with strong thermal contrasts they are of three different types. They are characterized by: (1) a sharply marked midday peak during the cool season; (2) a morning and a late-afternoon peak during the hot season; and (3) between these two extremes, a profile of daily activity curves that takes the form of a plateau changing progressively into one or the other of the two preceding types.

These two factors, areas and activity rhythms, obviously affect the possibilities of encounters between predators and tsetse flies. However, it is not known if the behaviour of habitual predators (such as asilids, dragonflies, bee-flies) and birds or bats, is similarly modified by the same climatic factors.

Tsetse flight is rapid and short; there-

[2] Recently developed Night Vision Devices based on the light-accumulation principle make possible accurate observations of nocturnal insect behaviour, even on dark nights (ed).

fore, only the quickest predators are likely to catch these insects. The short duration of the predatory act is in itself enough to explain the scarcity of observations. At times of major dispersal, *Glossina* spp. are exposed to a greater number of insectivorous birds and strong flying insects, such as dragonflies. When their movements are limited to more restricted habitats, they become more vulnerable to predators having a small radius of activity.

The capture of tsetse flies while they are taking a blood meal (a type of predation about which little has been published) is easier, for they are then not very sensitive to external stimuli. Southon observed dragonflies capturing tsetse from men, and Ford recorded the capture of feeding *G. tachinoides* by a hymenopteran (Sphecidae, *Oxybelus lamellatus*).

Estimation of the Importance of Predation

Close association of predators and tsetse inevitably increases the chances of the latter's capture. One can therefore expect increased predator incidence among tsetse populations when their density increases, as is the case during the hot periods of the year when the areas of activity of the two groups most nearly coincide. As an ecological generalization, the number of prey killed tends to increase with their density. Although it is sometimes true that an increased concentration of prey species gives them a measure of protection against their enemies, this does not seem to be the case for *Glossina* — in fact, it seems to be quite the contrary.

It is difficult to evaluate predation rates. The study of population densities of tsetse flies shows seasonal variations associated with both climatic factors and intrinsic and extrinsic biotic factors (such as parasites or predators, whose effects combine at certain periods of the year to reduce tsetse numbers). In the lower Chari River Valley, for example, the *G. tachinoides* populations show a marked decline caused by the combined action of drought and parasites (*Thyridanthrax*) on puparia and of predators (hersiliid spiders and eventually asilid Diptera) on adults (Gruvel 1974b). But how, other than by making direct observations, are we to determine the action of a particular predator on puparia or adults? In this connection, it must be appreciated that the effect of a predator on its prey is not always obvious. Moreover, predation is undoubtedly accentuated by the physiological condition of the prey; thus adults in states of rest during digestion, or of torpor under the influence of heat, are more vulnerable than those only temporarily settled. On the other hand, it must also be noted that, during studies of the resting places of *G. tachinoides*, flies marked with a coloured spot on the thorax comprised the major percentage of the victims of hersiliid spiders (Gruvel 1974a). Tsetse flies so marked are evidently more easily detected by predators, and thus more vulnerable to them. This is an important point that must be taken into account in all controlled experiments dealing with predation upon *Glossina*.

The estimation of predation rates attributable to each of the various predators is therefore extremely difficult. The introduction of mathematical models in such studies is indeed desirable, as they should lead directly to a better understanding of the reciprocal actions of factors governing tsetse ecology. However, because of the paucity of available information (particularly on tsetse-predator as well as tsetse-parasite relationships) it is necessary to keep referring back to observations from nature, pending the perfection of appropriate models.

Role of Predators in Biological Control

Although a few authors have attempted to estimate the capture of tsetse puparia or adults by ants or spiders, the assessment

of predation is usually purely qualitative, depending on direct observations of captures. Under such circumstances only the most abundant predators of adult tsetse flies are taken into proper consideration. We shall, of course, consider only active predators searching for their prey (dragonflies, ants, asilids, hersiliid spiders, etc.) as opposed to rarely encountered passive predators (*Nephilia* spiders). We may thus consider more particularly a few predators, such as asilids, ants, and Hersiliidae, the effectiveness of which has been abundantly confirmed during certain periods of the year. Their polyphagy is an adverse factor that may reduce their efficiency for biological control. To increase their efficiency in a control program, it would be necessary either to increase their numbers, or to protect them from all external dangers.

An increase in the number of predators in a given zone may be achieved by a massive introduction. However, this type of intervention is possible only if there is a nearby rearing centre producing large quantities of the most appropriate predator or predators. Present shortcomings in our knowledge of the biology of candidate predators precludes any successful rearing in the foreseeable future.

The conservation of predators in their natural habitat deserves some attention. Although we know little about this with respect to the predators under consideration, it is possible that they in their turn are preyed upon by natural enemies that contribute to limiting their numbers. For example, the asilid *Dysmachus prescipennis*, whose role in the maintenance of the insect fauna and as an auxiliary to agriculture was studied by Weinberg (1968), lies at the middle of a food chain between its own prey and spiders.

But the most interesting aspect of the execution of a program of integrated control is the use of synthetic chemical pesticides. It has been shown that aerial applications of DDT and HCH, if not pressed to the point of extinction of *Glossina*, may cause higher mortality among some of their parasites than among the flies themselves. After the cessation of spraying, the result is an abnormal increase of tsetse populations.

True, ground-based applications of residual insecticides are selective, being restricted to the preferred resting places of tsetse and aimed at their eradication. However, incomplete success of spraying might allow their natural enemies, such as predators, to complete destruction of the tsetse population. To what extent are these predators affected by localized sprayings of tree-trunks and low branches? Theoretically, only the fauna belonging to the biotope is killed by the insecticide. Thus, hersiliid spiders seem to be the most vulnerable; whereas, asilids, located at the tips of untreated isolated stems, have every chance of escaping the toxicant. At least insofar as the asilids are concerned, this is what actually happens. Indeed, a few months after a DDT application to *G. tachinoides* sites in the lower Chari River Valley, the endemic asilids could be taken in large numbers. Very often beyond the reach of the insecticide because of their favoured ambush sites, and especially because they disperse over a larger area than that actually treated, they can maintain or easily rebuild their populations. In contrast, spiders cease to be prevalent on the tree trunks, probably because of a combination of their being killed by the insecticide or some future microbial pathogen, vulnerable hot-season prey, *Glossina*, due to spray mortalities.

The nonspecificity of any broad-spectrum tsetse control agent, whether a pesticide or some future microbial pathogen, demands the most careful application methodology if the vector's natural enemies are not to be harmed at the same time.

Are living organisms capable of extracting from the habitat tsetse puparia or adults without actually feeding on them, to be included among our predators? Without dwelling on this possible extension of the classic definition, one must nevertheless mention man's role, sometimes far from

negligible, as a "predator" on tsetse. Field captures, although important, do not always affect the whole of the population. In some cases, however, they have significant consequences:

(1) The densities of *G. tachinoides* populations calculated monthly during several years in the same habitat (capture-recapture method) show seasonal variations according to a remarkably regular rhythm and at levels that are demonstrably similar from one year to the next. Following the extraction of nearly 5000 puparia from a particular habitat in a few days, the population declined over the next year to one third of what it was previously (Gruvel 1974b).

(2) When carried out on a large scale, the collection of puparia and adults may lead to the extermination of small isolated populations (Glasgow and Duffy 1961).

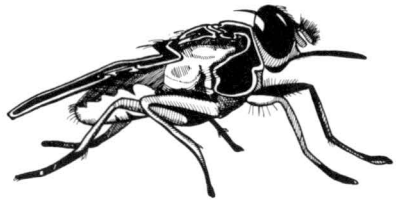
The extraction by man of large quantities of puparia and adults from an isolated habitat may prove an important aid (worthy of consideration in reducing population sizes) at least toward the reduction of tsetse species living in savannah forest galleries.

In conclusion, *Glossina* spp. have more natural enemies than many other vectors. A large number of these enemies contribute to the natural control of tsetse populations. Predators on tsetse are many and varied. Whereas a few are really efficient, the predatory action of others is no more than an entomological curiosity.

Our lack of knowledge of the biology and ecology of the predators is particularly striking, and justifies the interest that should be focused on the study of elements likely to be useful in biological control. Taking into account our present ignorance of most of the points raised, a vast research program would be necessary to provide the basis for a proper appraisal of the matter. In any case, an inventory of predators must be established, accompanied by an accurate key to the identification of species.

Comprehensive studies of the ecology of these predators should be undertaken, covering their habitats, food preferences, longevity, breeding periods, and population dynamics. This research must be undertaken bearing in mind the relationships between predators and tsetse, in order to arrive at estimates of predation rates and of their variations — estimates from which it will be possible to judge the real efficiency of the predator under study. Unfortunately, reliable methods for such estimates have yet to be developed! At the same time, it cannot but be useful to study the ecology, neglected until now, of certain *Glossina* spp., particularly those of the forest regions. Once this lack in our knowledge has been eliminated, it will be of primary importance to elucidate the factors inciting the predators to choose tsetse as prey, in preference to other organisms. We will then be able to capitalize on this information to obtain better control of the target insect.

The problems concerning the biological control of tsetse flies are essentially the same throughout their entire area of distribution. Investigators should therefore be encouraged to cooperate with one another on a more sustained basis, to facilitate the design of efficient methods meriting retention when a program of integrated control is established. — J. Gruvel.



Parasitoids

Much effort and expenditure have gone into various anti-tsetse campaigns involving bush-clearing, human population displacements, game control, fencing to prevent infected game moving into cleared areas, widespread use of insecticides, inspection and disinsection of vehicles to prevent reinfestation, etc. However, little has been attempted to date by way of biological control techniques.

Nevertheless during the course of studies on the biology and ecology of *Glossina* spp. in various parts of Africa, a wide range of natural enemies has been recorded; some fairly regularly, others only occasionally when reared from puparia.

Austen and Bagshawe (1914) suggested the introduction from other areas of muscoid fly parasitoids not already present in Africa, specifically mentioning *Spalangia*. In fact, several species of this genus already parasitize muscoids in Africa. Simpson (1918) referred to the possibilities of biological control in West Africa where he found puparial parasitoids of *Glossina* to be very rare. These general ideas were certainly worthwhile considering. They remain so today. Three main lines of approach commend themselves: (1) the artificial dispersal within Africa of parasitoids of *Glossina* to areas where they do not already occur; (2) the introduction into Africa of parasitoids attacking dipterous puparia elsewhere, and perhaps able to attack *Glossina* puparia successfully (both these approaches would involve the introduction into new areas of parasitoid species, with a view to their permanent establishment as part of the regulating mechanism of *Glossina* populations); and (3) the mass releases of selected parasitoids for the temporary suppression of tsetse populations.

However, only three serious attempts to use entomophagous parasites for tsetse control have been made, all involving *Syntomosphyrum* spp. There does not appear to have been any other similar major effort along these lines. This is perhaps not too surprising when the biology and ecology of *Glossina* are taken into account, and the lack of success of these initial trials is considered. In two of these instances there was certainly an increase in parasitism, and hence of destruction of *Glossina*, but in practical terms, the actual level of control achieved was insignificant.

Some general points emerge from a consideration of these examples. The selection of *Syntomosphyrum glossinae* Waterston in the first instance was understandably influenced by the fact that the parasite is easy to breed in the laboratory. The later experiment at Kikori (p. 62) was obviously an extension of the previous one in Malawi. It seems that very little (if any) effort was made to ascertain the suitability of *S. glossinae* for this purpose. The species was used because at that time other parasitoids appeared to be more difficult to manipulate. The bombyliids could not be bred because the adults damaged themselves in cages; whereas the mutillids had long life cycles and low reproductive rates (although it was later found that *Mutilla glossinae* Turner could be reared on *Sarcophaga* puparia).

Nash (1970b) has reviewed the control exerted on *Glossina* by natural enemies, whereas Heaversedge (1968b) gives accounts of parasitoids from Rhodesia.

There are several quite different aspects of the possible use of parasitoids against tsetse. In any detailed consideration of field release programs (whether for permanent establishment or temporary augmentation) we must be certain about the target species of *Glossina* and the ecological conditions under which the attempt is to be made.

A parasitoid selected for use against a relatively abundant riverine *Glossina* would have to have rather different characteristics than a parasite used in more open, possibly drier, country. In the first case reproductive capacity should, for example, be stressed, whereas in the latter, where the local *Glossina* is less abundant, searching capacity or longevity under arid conditions might be of equal or greater importance. Similarly, the effectiveness of different parasitoids might vary during particular seasons of the year. Possibly, combinations of parasitoids with different characteristics might be used to supplement one another. Mass-releases can be made either to introduce an effective species into an area where it does not already occur, or (as in the experiments with *Syntomosphyrum*) on a periodical basis with a view toward increasing the numbers and effectiveness of a parasitoid already occurring in the area.

Economically, the former is certainly preferable. Once established in an area, an effective parasitoid should not need periodic supplementation, and hence the costs of periodic releases are eliminated. Whichever method is adopted, but particularly with respect to periodic releases, there are problems associated with breeding extremely large numbers of a selected parasitoid in the laboratory (see Mass Rearing Using Animals for Feeding).

We shall consider all the parasitoids of *Glossina* so far recorded. It seems rather unlikely that others offering any promise for biological control remain to be discovered, in view of the widespread general investigations that have now been carried out in Africa. It is thus a question of selecting the most promising parasitoids from this list. The species appearing to warrant first consideration in this regard are described in greater detail than the others. A second approach, the importation into Africa of non-tsetse parasitoids for use against *Glossina*, is also considered.

None of these aspects presents a simple or particularly promising solution to the tsetse problems. With the vast area covered by ecologically different species of *Glossina* there is more than one "problem." It is impossible that any single attempt at biological control will solve them all. However, until further detailed investigations have been carried out, we shall advance no further.

Syntomosphyrum spp.

Systematics and Distribution

The genus *Syntomosphyrum* is a member of the hymenopterous family Eulophidae. *S. glossinae*, first found parasitizing puparia of *G. f. fuscipes* (formerly considered as *G. palpalis*) from the shores of Lake Victoria, was described by Waterston (1915a, b). For some years, this was considered to be the only species of *Syntomosphyrum* parasitizing *Glossina* puparia, although the existence of a form of the species with white-clubbed antennae, distinct from the originally described dark-clubbed form, was recognized by Waterston (1916). Saunders (1960a) found that cross-matings between the two forms resulted in the production of all-male progeny. These were presumed to have developed from unfertilized eggs, and he concluded that the two forms should be regarded as separate species. The white-clubbed form was described as *S. albiclavus* Kerrich (1960).

The distribution of the two species of *Syntomosphyrum* has been examined by Saunders (1960a) and (in tabular form) by Potts (1970b). Distribution records are summarized in Table 2. *S. glossinae* has been recorded from a number of *Glossina*

Table 2. *Glossina* parasitoids by host and by country.

	morsitans	*morsitans* group: *austeni*	*morsitans* group: *swynnertoni*	*pallidipes*	*fusca* group: *brevipalpis*	*fusca* group: *longipennis*	*palpalis* group: *tachinoides*	*palpalis* group: *palpalis*	*Glossina* spp.	Senegal	Chad	Sierra Leone	Ghana	Nigeria	Cameroon	Zaire	Sudan	Ethiopia	Uganda	Kenya	Tanzania	Zambia	Malawi	Rhodesia	South Africa (Zululand)	Mozambique	Botswana	South West Africa (Namibia)
Mutillidae																												
Mutilla glossinae Turn.	+			+	+																	+	+	+				
M. benefactrix Aust.	+																						+	+				
M. auxiliaris Turn.	+	+		+	+																				+	+		
Bombyliidae																												
Thyridanthrax abruptus Lw. (=*T. lineus* Lw.)	+		+	+	+	+		+?	+							+	+		+	+	+	+	+	+	+	+	+	+
T. salutaris Aust.	+																				+		+	+				
T. argentifrons Aust. (= *beckerianus* Bezzi)	+		+	+			+	+?		+	+	+	+?	+	+						+			+?				
T. transiens Bezzi	+																			+			+	+	+	+		
T. beneficus Aust.	+																							+		+		
T. lugens Lw.	+	+	+	+	+	+														+	+			+	+			
T. alliopterus Hesse		+	+	+	+	+															+	+				+		
T. burtti Hesse		+																			+					+		
T. brevifacies Hesse		+		+		+																				+	+	?
T. lloydi Austen.	+								+					[+] error				+				+	+		+			
Petrorossia spp.																												
P. angustibasalis Hesse	+																								+			
P. hesperus tropicalis Bezzi	+																								+			
Conopidae																												
Stylogaster westwoodi Swinh. (once only)	+																		+									
Eupelmidae																												
Eupelmella tarsata Wtrst.	+																							+		+		
Anastatus viridiceps Wtstn.	+	+						+						+											+		+	
Chalcididae																												
Haltichella edax Wtrst.	+																						+					
Stomatoceras micans Wtrst.	+			+			+							+						+	+	+		+		+		
S. exaratum Wtrst.	+																						+	+				
S. schulthessi Ferri.	+						+							+									+	+				
S. diversicornis Kirby.	+																							+				
Brachymeria amenocles Wlk. (= *B. varipes* Wlk.)	+						+						+											+				
Dirhinus sp.	+																											
D. giffardii Silv.	+			+				+					+?										+	+				
D. inflexus Wtrst.	+												+															
Eulophidae																												
Syntomosphyrum glossinae Wtrst.	+	+		+	+		+	+	+		+	+		+					+	+	+		+	+				
S. albiclavus Kerrich	+			+				+?												+	+	+	+	+	+	?		
Braconidae																												
Coelalysia glossinophaga Turner	+												+															
Bethylidae																												
Prolaelius glossinae Turn. & Wtrst.	+																						+?		+	?		
Perilampidae																												
Perilampus ruficornis F. (= *P. violaceus* F.)	+													+														
Ceraphronidae																												
Conostigmus rodhaini Beq.														+?		+												
Diapriidae																												
Trichopria capensis robustior Silv.	+			+	+											+			+					+				
T. lewisi Nixon				+	+															+								
Abrothropria lloydi Ferr.	+																				+?			+?				

spp. from Senegal, Liberia, Nigeria, Chad, Uganda, Kenya, Tanzania, and Malawi. It can therefore be presumed that this species is generally distributed in Africa's flybelt. *S. albiclavus* appears to be of rather more restricted distribution, having been recorded with certainty only from Kenya, Tanzania, Malawi, Zambia, and Rhodesia. There is uncertainty about the true status of many early records of *S. glossinae* and of the species to which "*S. glossinae*" from Mozambique (Dias 1961) and South Africa, notably Zululand, (Fiedler and Kluge 1954), should be attributed.

Life Cycle and Ecology

Virtually nothing is known about the life cycle of *Syntomosphyrum* spp. in the field. Experimentally, females will oviposit in the puparia of a wide variety of the higher Diptera, including species of *Musca, Dacus, Lucilia, Chrysomyia, Sarcophaga, Calliphora, Phormia,* and *Stomoxys.* It will even parasitize puparia of *Drosophila,* although these, because of their small size, are inadequate hosts (Saunders 1961). The record of *S. glossinae* from cockroach oothecae (Nash 1955) is erroneous (Jordan 1956). It is not known which hosts, other than *Glossina* spp., are attacked in nature. Mature females drill through the puparium of *Glossina* and oviposit within it. Saunders (1961) indicated that as the female of *S. albiclavus* normally kills the pupa within the puparium before ovipositing, it is a "refined predator" rather than a parasitoid in the strict sense of the term. Apparently, the puparium is attacked at any stage of its development (Harris 1930). The statement by Nash (1933a) that the larva of *Glossina* can be parasitized before it has burrowed into the ground was questioned by Saunders (1961) on the grounds that *Syntomosphyrum,* like many other parasitoids, needs to oviposit into a "subpuparial space" (i.e. between the puparium, in the strict sense, and its contents) for successful parasitization.

The development of the immature stages of *S. albiclavus* within puparia of *Lucilia sericata* (Mg.) has been described by Saunders (1960b). Puparia were exposed to females of *S. albiclavus* when 6-h old, and were then incubated at 25 °C. Eggs were deposited on the surface of the larva or pupa within the puparium. The resulting immature stages occupied the space between the pupa and the puparium.[3]

The number of eggs laid depends on the size and age of the female. Under favourable conditions, some 40–50 eggs are laid. These hatch about 48 h after oviposition. The larvae are translucent and apodous. Each of the first three larval instars has a duration of about 1 day. The fourth (final) instar lives for some 5 days. The prepupal and pupal stages last for about 2 and 10 days, respectively. After the adults emerge they remain within the host puparium for another day or so before biting a small hole in the puparium and escaping. Copulation occurs just after emergence from the host. Females mature in about 3 days and are then able to parasitize new hosts. The sex ratio of emerging parasitoids is usually about 1 male to 6 females. Virgin female *Syntomosphyrum* produce only male offspring.

In the laboratory, *Syntomosphyrum* can be hyperparasitic on *Mutilla* within the puparium of *Glossina,* a situation first observed by Lamborn (1916). Lamborn (1925) found no difficulty in producing both parasitic and hyperparasitic broods.

In nature, the adults have a limited ability to penetrate soil, which probably means that many puparia of *Glossina* are immune to attack. Nash (1933a) found that the ability to penetrate soil depended upon the soil type. In tightly packed dry sand the insects could not reach puparia deeper than 2.5 mm. At the other extreme, they

[3] "Puparium" is used here in its strict sense as the shell derived from the final larval skin enclosing the developing pupa or phorate adult. Elsewhere it is used loosely to describe the puparium and its contents.

Table 3. Rates of parasitization by *Syntomosphyrum* spp. of puparia of *Glossina* in nature.

Species	No puparia examined	% parasitized	Locality	Authority
G. m. morsitans	2340[a]	0.04	Malawi	Lamborn (1916)
G. m. morsitans	1700[b]	2.4	Malawi	Lamborn (1916)
G. m. morsitans	1367	0.2	Malawi	Lamborn (1925)
G. m. morsitans	7440	0.4	Rhodesia	Chorley (1929)
G. m. centralis	5168	0.2	Tanzania	Nash (1933a,b)
G. m. centralis	2185	0.2	Tanzania	Potts (1933)
G. f. fuscipes	203	1.5	Lake Victoria	Fiske (1920)
G. austeni	26543	0.1	Mozambique	Dias (1961)
G. pallidipes	30331	0.3[c]	Rhodesia	Heaversedge (1969a)

[a] Live puparia.
[b] Empty cases.
[c] Mean of a series of percentages for two areas and two years.

could reach puparia beneath 15 mm of dry humus, and 38 mm of dry leaves.

Difficulties experienced by parasites in locating buried puparia may at least partly account for the low rates of parasitization of *Glossina* puparia in the field. Despite the ease with which dipterous puparia can be parasitized by *Syntomosphyrum* in the laboratory, natural rates of parasitism higher than 3% have not been recorded. Available data are summarized in Table 3. This parasitoid is apparently absent from many areas. In others, rates of parasitization of *Glossina* puparia are even lower than those given in Table 3. This can lead to a false assumption of its absence from a particular region. Thus *Syntomosphyrum* was unknown from West Africa until small numbers were found in puparia of Nigerian *G. palpalis* (Nash 1947).

Attempted Use in Biological Control of *Glossina*

Syntomosphyrum is the only parasitoid which has, to date, been used in an attempt to control *Glossina* by biological methods. Lamborn (1916, 1920, 1922) carried out laboratory investigations into the biology of *Syntomosphyrum*. Saunders (1961), who suggested that Lamborn was working with the species now known as *S. albiclavus*, was impressed with this parasitoid's longevity, short life cycle, high fecundity, and the ease with which it could be bred in a variety of dipterous puparia.

Lamborn's work was quoted at considerable length by Austen and Hegh (1922), who were much influenced by Dr G.A.K. Marshall's preoccupation with the recording by Lamborn (1916) of *Syntomosphy-*

Table 4. Field releases of *Syntomosphyrum* spp.

	Locality	Natural incidence in puparia (%)	No. of parasitoids released	Incidence in puparia after releases (%)	Authority
G. m. morsitans	Malawi	0.2	277000	6.8	Lamborn (1925)
G. m. submorsitans *G. tachinoides*	Nigeria	0	"large numbers"	0	Lloyd et al. (1927)
G. m. centralis	Tanzania	0	3.5 million	0.8	
			2 million+	4.0–9.9	Nash (1933a)
			8.25 million+	0.8–1.6	
G. f. fuscipes	Tanzania	?	519000	5	Lloyd (in Swynnerton 1936)

Female Syntomosphyrum glossinae (left), female Mutilla glossinae (right). The scale line indicates 1 mm (from Buxton 1955).

rum hyperparasitism of mutillids, which led to his conclusion (quoted from Austen and Hegh) that "There seems little doubt now that this species is harmful, being a hyperparasite of *Mutilla glossinae*." A little later in their monograph Austen and Hegh (1922) omitted from their list of the then-known *Glossina* puparial parasites "*Syntomosphyrum glossinae*, Wtst., which, as already stated, has been proved to be a hyperparasite of *Mutilla glossinae*, and therefore harmful instead of beneficial." Nevertheless, the first field campaign, involving the release of insects reared in captivity, was undertaken by Lamborn (1925). The results of this and subsequent campaigns are summarized in Table 4. Exposing puparia of *Sarcophaga* to *Syntomosphyrum* in the laboratory, Lamborn obtained high rates of parasitism. For protection from predators, the parasitized puparia were enclosed in sealed lengths of bamboo and placed in tsetse habitats. A small hole was drilled in the tube to allow the parasitoids to emerge. The field releases were carried out in about 110 km^2 (42 miles^2) of a peninsula in Lake Nyasa; in June–October 1923 some 277 000 parasitoids were released. Whereas the natural incidence of *Syntomosphyrum* before the release was very low (some 0.2% of 1367 puparia were attacked), the incidence in the first 3 months of 1924 had increased to 6.8% of 516 tsetse puparia examined. No further releases were carried out. Eight months later, the parasitoid's incidence had returned to the original low level (Buxton 1955).

The second attempt to use *Syntomosphyrum* to control *Glossina* was made near Sherifuri in northern Nigeria. Some of Lamborn's original stock of *S. albiclavus* had been sent to England and from there consignments of parasitized blowfly puparia were sent by surface mail to Sherifuri, where *S. albiclavus* did not occur naturally. A large stock was built up from the few survivors, and "large numbers" of the parasitoid were released in an area where *G. m. submorsitans* and *G. tachinoides* were breeding actively. However, parasitized tsetse puparia were not recovered subsequently Lloyd et al. 1927).

The largest-scale release program was undertaken at Kikori, Tanzania, by Nash (1933a). Before releases were begun, specialized techniques for rearing and releasing *Syntomosphyrum* (the species is uncertain) were developed. Parasitoids were bred from puparia of *Chrysomyia*, each parasitized puparium producing an average of 70 parasitoids. Precautions had to be taken to guard against parasitization of the *Chrysomyia* puparia by the hymenopteran *Trichopria* sp., which does not attack *Glossina* puparia, and to prevent ants attacking the parasitized *Chrysomyia* puparia after they were released in the field. Following the development of satisfactory techniques, releases were made along the base of an escarpment in *Brachystegia microphylla* woodland,

where the parasitoid either did not occur or was exceedingly rare. Releases began in March 1931. By early December 1931, it was estimated that some 3.5 million *Syntomosphyrum* had been released. A sample of 254 puparia of *G. m. centralis* was collected, and it was found that 0.8% were parasitized by *Syntomosphyrum*. The remainder either produced a tsetse or were dead through other causes. The next month, 2.0 million more parasitoids were liberated. In one area 4.0% of 50 puparia, and in another, 9.9% of 171 puparia were parasitized. During the next 4 months a further 8.25 million *Syntomosphyrum* were liberated. At the end of this period, rates of parasitism had fallen to 0.8% of 124 puparia and 1.6% of 489 puparia. Throughout the experiment, no *Syntomosphyrum* were found in two control areas. Clearly, the release of 13.75 million parasitoids, albeit at extremely low cost, had achieved little.

Nash (1933a) attributed the failure of his release program to the inability of *Syntomosphyrum* to penetrate the sandy soils in the experimental area. In an attempt to overcome this problem, Lloyd released Kikori-stock *Syntomosphyrum* into a habitat where *G. fuscipes* — then identified as *G. palpalis* — was breeding in humus (Riamugasire Island, Lake Victoria). In 1 month, some 519 000 *Syntomosphyrum* were released: only 5% of the tsetse puparia became parasitized. It was suggested that the humus was too moist for significant numbers of the parasitoid to penetrate (Swynnerton 1936).

Glasgow (1963) summed up the attempts to use this parasitoid: "Looking back, it seems that when it was decided to undertake these experiments, too much weight was given to the ease with which *Syntomosphyrum* can be bred artificially, and too little to the fact that it has never been found abundant in nature." This is probably a fair comment, but the possibility of using the species under particularly suitable conditions should not be completely excluded. There does remain some slight possibility of it being effective against tsetse breeding for at least part of the year in loose, dry, friable humus.

Mutilla spp.

Systematics and Distribution

Three species of the hymenopterous family Mutillidae (Turner's *Mutilla glossinae, M. auxiliaris* Turner 1915, 1916, 1920, and *M. benefactrix* Austen) have been reared from puparia of *Glossina*. This nomenclature is retained, to avoid confusion with species referred to in the literature. However, Brothers (1971) suggested that the first two species should be transferred to a new genus *Chrestomutilla* and the third species to *Smicromyrme*. *M. glossinae*, a much commoner parasitoid of *Glossina* than either *M. auxiliaris* or *M. benefactrix*, is the only species that has been studied in any detail both in the laboratory and in the field.

The three species of *Mutilla* have a limited distribution as parasitoids of *Glossina*, none being found outside southern Africa. Available records are summarized in Table 2. *M. auxiliaris* was present as a parasitoid of *Glossina* in Zululand, South Africa (Fiedler and Kluge 1954) before tsetse were eradicated from the area (du Toit 1954). The current status of *M. auxiliaris* in this area is unknown. Most records of *Mutilla* spp. from tsetse are from Zambia, Malawi, and Rhodesia, where high parasite rates have been recorded from *Glossina* puparia.

Life Cycle and Ecology

Although adult female *M. glossinae* are apterous, they can be carried some distance through the air by the male during copulation. Little is known of the biology of the species in nature, but a number of laboratory studies have been undertaken. Adult female *Mutilla* fed on jam, will readily oviposit in puparia of *Glossina*. Much less readily, they will also do so in

puparia of *Sarcophaga* (Lamborn 1925; Chorley 1929). Female *M. glossinae* have three ovarioles in each of two ovaries. The oocytes in three of the ovarioles are ready for deposition shortly after emergence (Heaversedge 1969c). Newly parasitized tsetse puparia generally contain two or three mutillid eggs and only rarely a single egg. As three eggs mature at once, it seems likely that all are deposited within one puparium. Some days necessarily elapse before more oocytes have matured and parasitization can be resumed. Of the eggs laid in the tsetse puparium, one must develop at the expense of the others — it is unknown for more than one mutillid to emerge from a puparium. The larva was briefly described by Lloyd (1916).

At 25 °C, Heaversedge (1969c) found that the egg stage lasted some 4–5 days, and the larval stages an additional 8–9 days. Prepupae were present until 20–24 days after the puparia were parasitized. The puparial stage lasted a further 10 days or so before emergence of the adult. Adults left the puparium after about 46 days. After the departure of the adults a previously parasitized puparium can readily be distinguished by the presence of the mutillid's empty, yellow, silky cocoon. The size of the adult parasitoid depends on the size of the puparium attacked. *Mutilla* spp. from puparia of *G. pallidipes* are consistently larger than those from *G. morsitans* (Heaversedge 1968a) and from *G. austeni* (Fiedler and Kluge 1954). Under field conditions, the duration of the immature stages may considerably exceed the period reported by Heaversedge (1969c). From Malawi, 2.5- to 4-month periods have been reported.

The sex ratio of *M. glossinae* emerging from puparia of *Glossina* is usually about 6 females: 1 male. For instance, Chorley (1929) recorded 15% males in a total emergence of 1176 mutillids in Rhodesia. In Zambia, Dollman (1916) obtained only one male from a total of 64 mutillids from parasitized puparia of *G. morsitans*. Unbalanced sex ratios, common in the Hymenoptera, also occur in *Syntomosphyrum*. They are usually the result of a form of parthenogenesis (arrhenotoky), haploid eggs developing into males and diploid eggs into females. Heaversedge (1969d) concluded from 2-year-long studies in Rhodesia that sex ratios found in *M. glossinae* definitely indicated arrhenotoky. In each of 3 years, the proportion of male mutillids emerging from tsetse puparia was lowest during the summer (10–20 males/100 females) and highest during the winter (50–60 males/100 females). The reasons for these differences are unknown. Heaversedge (1969d) suggested that they could be associated either with reduced activity of the parasitoid in colder weather (reducing the probability of male-female encounters, and consequently increasing the number of unfertilized females producing male progeny), or with some seasonal factor affecting the fertilization of eggs in the female. When tsetse puparia were exposed to *M. glossinae* in the laboratory by Lamborn (1916), 58 males and 115 females were produced. This suggests that under conditions of stress it is possible that fewer eggs are fertilized and abnormally large numbers of males are produced.

Natural rates of parasitism of puparia of *Glossina* by *M. glossinae* have been recorded by numerous investigators. Caution has to be exercised in the interpretation of the data: some investigators derived their proportions parasitized from the total number of puparia collected, others from the total insects (tsetse + parasitoids) that emerged, all unproductive puparia being excluded. Heaversedge (1969b) concluded that the latter is the most satisfactory method, as it is often difficult to determine whether or not a puparium has contained a parasitoid. In general, this is a reasonable opinion. However, it must also be recognized that values derived for percentage parasitism in this way will be overestimates for some species of parasitoid. Some parasitoids (excluding *Syntomosphyrum* spp. but including *Mutilla* spp. and especially *Thyridanthrax* spp.) are enclosed within the puparium for longer periods

than the developing tsetse fly. There will, therefore, always be a bias in favour of parasitized whole puparia in any one sample.

In view of these difficulties and marked differences in rates of parasitism from year to year, season to season, and place to place, it is not meaningful to tabulate data that have been obtained for *M. glossinae*. However, certain salient features may be emphasized. The parasitoid is much less widely distributed than *Syntomosphyrum* spp., although, where it does occur it parasitizes a much higher proportion of tsetse puparia. In Malawi, Lamborn (1915a, 1916) recorded rates of 3.5 and 6.3% from 9762 and 7731 puparia of *G. m. morsitans*, respectively. He bred 41 *M. glossinae* from one small sample of 85 puparia.

Evidence for seasonal variations in the rate of parasitism has been produced from Rhodesia. Chorley (1929) found that rates of parasitism of weekly batches of at least 300 *G. m. morsitans* puparia were lowest in the cold, dry months (0.5% of total emergences of tsetse and parasitoids). In the hot, dry months the weekly rate was never less than 14% and often over 20% (42.7% in one week). As the rains began, the rate fell to 6–12% and observations ceased. Chorley's observations were carried out in the Umniati flybelt. Some years later, Heaversedge (1969b) obtained rather more detailed results from two other areas in Rhodesia — Lusulu and Kariba. In both areas she found that rates of parasitism were generally lowest in both cold, dry and hot, dry months and highest in warm, wet months (monthly rates varied from 1.9 to 18.9%). Heaversedge took these studies a stage further, recording rates of parasitism in different types of breeding sites utilized by *G. m. morsitans* at various times of the year. Significantly higher levels of mutillid parasitism were consistently found in puparia from "out of season" sites than from those sites most utilized at a particular time of year. This finding was almost certainly an artifact, resulting from the longer time spent within the tsetse puparium by *M. glossinae* than by the developing tsetse fly — after the healthy tsetse hatched only the parasitized puparia remained (Jack 1939).

Heaversedge (1969a) also obtained data on the rate of parasitism of puparia of *G. pallidipes* by *M. glossinae*. In each of 2 years, highest rates of parasitism were recorded in hot, dry months (14% of 838 puparia in one year and 11.7% of 273 puparia in another). Levels of parasitism by *M. glossinae* were generally lower for *G. pallidipes* than for *G. m. morsitans* (see above), although the puparia were collected from the same sites.

Potential for Use in Biological Control of *Glossina*

The release of laboratory-reared *Mutilla* into natural populations of *Glossina* has not yet been attempted. Lamborn (1925) commented on the low reproductive capacity of *Mutilla* spp., which may be largely attributable to the restrictions of its reproductive morphology and to the time taken by the immature stages to develop (Heaversedge 1969c). Both investigators considered that the chances of breeding enough mutillids to achieve some measure of control of a tsetse population might be remote. Certainly a search would be required to determine whether more readily available hosts than puparia of *Glossina* might be fully acceptable to *Mutilla* spp.

If problems associated with rearing large numbers of *Mutilla* spp. could be overcome, these parasitoids could clearly become useful biological control agents. They can certainly produce relatively high rates of natural parasitism. It has been suggested (Nash 1969; IDRC 1974) that it might be profitable to introduce *M. glossinae* into West Africa,[4] in particular to drier regions where breeding by *G. m. submorsitans* is seasonally concentrated in restricted vegetation types.

[4] The IDRC is supporting a project along these lines with a grant to The Commonwealth Institute of Biological Control.

Bombyliidae

Two genera of bombyliids *Thyridanthrax* (= *Exhyalanthrax*) and *Petrorossia* are recorded as parasitoids (entomophagous parasites) of different species of *Glossina*, all from puparia.

Thyridanthrax[5]

Systematics

This genus of bombyliids includes numerous species, several of which occur in temperate as well as in tropical regions. *Thyridanthrax* spp. do not appear to be highly specific, parasitizing not only various dipterans, but also some acridids.

Thyridanthrax was first recorded from *Glossina* in 1912 (Lamborn 1915b), when R.W. Jack reared *T. abruptus* from puparia of *G. morsitans* collected in Rhodesia. In 1913 Lloyd (1916) reared from *G. morsitans* a parasitoid duly described by Austen (1914) as *Villa lloydi*. Subsequently transferred to *Thyridanthrax* (see Hesse 1956), this bombyliid was also recorded from *Glossina* by Waterston (1915a, b). *Thyridanthrax* spp. were recorded by Austen (1929) from *G. morsitans: T. argentifrons* (= *beckerianus*), *T. beneficus, T. salutaris*, and *T. transiens*. Similar observations were made by Lester (1931) and Nash (1933a). There were later records from various species of tsetse by Hesse (1956), Fiedler and Kluge (1954), and Fiedler et al. (1954).

Nearly all these reports are from southern or eastern Africa; reports on *Thyridanthrax* from West Africa are scarce. However, Lester (1931) and Taylor (1932) recorded *T. argentifrons* from Nigerian *G. m. submorsitans*, and more recently Gruvel (1970a, b; 1974a, b) has carried out detailed studies in Chad on parasitism of *G. tachinoides* by *T. beckerianus*.

Ten different species of *Thyridanthrax* from *Glossina* spp. are currently recognized: *T. abruptus; T. alliopterus; T. beckerianus*, (= *T. argentifrons*); *T. beneficus; T. brevifacies; T. burtti; T. lloydi; T. lugens; T. salutaris*; and *T. transiens*. Three other species as yet not definitely identified also parasitize *Glossina*, one from Tanzania (under the name *T. argentifrons*) and two from Nigeria.

Identification of some of these bombyliids is difficult, due to individual variations that cannot be sufficiently studied because of the limited material that is presently available. Hesse (1956) wrote:

"Owing to the remarkable uniformity of the colour pattern and arrangement of the hairs and scaling in species of this genus, as well as the difference in the wing infuscation between males and females and individual specific variability which certain species show, it is very difficult and sometimes impossible to distinguish the various species and to recognize the subtle specific differences which separate them, especially if a long series of any one species be not available. This tendency to vary both sexually and individually and also specifically has caused confusion of species and the redescription of forms, races or varieties of some species under different names by authors who had only a few specimens at their disposal."

According to Hesse, two *Thyridanthrax* species have been confused under three different names: *T. abruptus, T. lugens*, and *T. lineus*.

Specimens identified as *T. lineus* are, in fact, males of *T. abruptus*. Others determined as *T. abruptus* actually belong to other species such as *T. lugens*. Commenting on Hesse's findings, Dyte (in Nash 1970b) stated that old records of *T. lineus* refer to *T. abruptus* and that those of *T. abruptus*, other than references by Fiedler and Kluge (1954), Hesse (1956), and Greathead (1967), probably refer to other species.

Gruvel (1974b) commented that *T.*

[5] Many of the species of *Thyridanthrax* have recently been transferred to *Exhyalanthrax*, but to avoid still further confusing the issue the old name will be used here.

argentifrons Austen 1929, must by priority cede precedence to *T. beckerianus* Bezzi 1924. There is still some doubt as to whether all specimens referred to earlier as *T. argentifrons* Becker 1910, are not in fact *T. beckerianus* Bezzi. However, for simplicity we will consider them synonymous. François (1972) synonymized *T. argentifrons* and *T. argyrolophus* (Hesse) with *beckerianus*. This clearly stresses the need for an up-to-date revision of the genus *Thyridanthrax*, which Dyte (in Nash 1970b) has proposed to undertake.

Distribution

Except for *T. beckerianus* (= *argentifrons*), recorded from West Africa, all other species of *Thyridanthrax* parasitizing *Glossina* are from East and southern Africa. Perhaps this only reflects the fact that comparatively fewer ecological investigations have been undertaken in West Africa. The general geographical distribution is evident from Table 2, which also gives records of *Thyridanthrax* from different species of *Glossina*. This table was compiled from a number of sources. It includes conflicting records, and cannot be regarded as entirely accurate. Other synopses are given in Nash (1970b) and Jenkins (1964).

Ecology

Analyses of the incidence of *Thyridanthrax* spp. in field collections of *Glossina* puparia have indicated important variations between different areas and different seasons of the year.

In 1930, Nash (1933a) reported 74 *T. abruptus* emerging with 764 *G. morsitans* (i.e. 9.7% parasitism) from a collection made at Kikori, Tanzania. Figures for *T. beckerianus* (= *argentifrons*) are more common. Taylor (1932) at Gadau, northern Nigeria, reared 47 *Thyridanthrax* from 137 346 puparia of *G. morsitans* (0.27%). In the same locality he obtained 694 parasites from 106 000 *G. tachinoides* puparia (0.66%). On the other hand, he obtained no parasites from 7500 puparia from the River Benue area, Nigeria. In Ghana (then the Gold Coast), Simpson (1918) failed to find a single bombyliid in a very large number of puparia of both *G. morsitans* and *G. tachinoides*.

Taylor's observations indicated that parasitism was highest in the warmer dry months, March and April (with up to 2.3% parasitism in March), and lowest in October and November after the rains. These records most probably refer to *T. beckerianus*. Perhaps they are less significant than those provided by Gruvel (1975a) in Chad, following his 1965–1972 studies of the parasitism of *G. tachinoides* by *T. beckerianus*. Gruvel demonstrated pronounced seasonal variation, with fairly high parasitism in the warmer months, April (25%) and May (17.5%) and lower parasitism at other times, e.g. 9% in June, and 4% in February and March. Dias (1961) in Mozambique found 0.7% parasitism by *T. lugens* in 27 000 puparia of *G. austeni*. It may be added that Buxton (1955) pointed out that very large numbers are not in fact necessary to make valid statistical calculations.

Other *Thyridanthrax* species from different *Glossina* hosts of southern and eastern Africa are mentioned by several authors, with somewhat similar degrees of parasitism. Chorley (1929) obtained two species of *Thyridanthrax* from 588 of 7440 puparia of *G. morsitans* collected (7.9%). Nash (1933a), who examined 5168 *G. morsitans* puparia at Kikori, Tanzania, found three species of *Thyridanthrax* attacking 310 of them (6%). In the same general area (Potts 1933) reported 6.2% parasitism, and on a number of occasions recorded levels of over 20%.

Records of particularly high rates of parasitism may be understood by considering both time and space factors in the ecology of the host *Glossina* spp. and their parasites. Attacks on *Glossina* puparia are very much more likely where: (1) the oviposition sites of *Glossina* and *Thyridanthrax* coincide; and (2) the tsetse puparia are concentrated in restricted sites rather than

dispersed over a wide area. Nash's observations also indicate differences in parasitism according to vegetation types. In 1928 and 1929 he obtained 20 and 22% emergence of *Thyridanthrax* from *G. morsitans* collected from areas of the trees *Berlinia globifera* and *Acacia usumbarensis*, respectively. However, the rate fell to 2% in 1931, and no parasitized puparia were found in 1932. Nash attributed this to ecological changes at the level of the soil from which the collections were made. With respect to *G. tachinoides* in Chad, Gruvel described several habitat types in which he found *T. beckerianus*. He noted that the importance of this bombyliid as a puparial parasite is particularly evident in the *Morelia senegalensis* habitats — occupied by tsetse from the end of the cool season and throughout the hot one. Within the major habitat, specific biotopes may differ as regards host/parasite occurrence and incidence.

For *G. m. morsitans*, Heaversedge (1969b) stated that parasitism was higher when the puparia were collected during the warm season within holes in living trees or from animal burrows, than it was when collections were made from under fallen logs, leaves, or rocks. However, this does not apply only to bombyliids. It is pertinent to quote Glasgow (1963):

"It is rarely that one collects a sample of puparia and can be sure that it adequately represents all the sorts of places in which puparia exist; it is therefore always possible that although a given species of parasite is excessively rare in a collection of puparia, it may be less rare in puparia occurring in other types of site which have not been sampled."

One may say, in general, that the highest rates of parasitism occur in the hot or wet seasons for many host/parasitoid associations in various areas. This was noted in southern Africa by Fiedler and Kluge (1954) and Fiedler et al. (1954), and in Kenya by Hursey (1970). In Chad, Gruvel recorded 41% parasitism in April 1970. On one occasion Chorley (1929), carrying out weekly puparial collections in Rhodesia, reported that in a total emergence of 362 *Glossina* and parasitoids, *Thyridanthrax* comprised 64% of the catch. It was therefore, with every justification that Fiedler and Kluge ranked *Thyridanthrax* among the most important parasites of tsetse in Zululand, South Africa. A similar claim may be made for other regions.

One cannot leave this section on the incidence of *Thyridanthrax* in natural populations of *Glossina* without emphasizing the paucity of existing data. Moreover, such information as we have is seldom at all detailed, since few of the species recorded have been intensively studied over a lengthy period. Also, even in those species for which a reasonable amount of information is available, figures based on thousands or even tens of thousands of puparia mask variations due to seasonal changes, and there is an absence of an exhaustive knowledge of the comparative ecology of parasitoid and host.

It is only to be added that these bombyliids have a much wider range than *Glossina* spp., and are found outside the distribution limits of the latter. Several general habitat types are described below.

In Gruvel's studies of *T. beckerianus*, adults were more commonly seen at midday from 11:00–15:00 on hot sunny days. They fly about 30 cm above the ground, moving rapidly up and down with brief periods of rest on low-growing plants or on the ground. *T. abruptus* is heliophilous, having been observed by Lamborn (1915b) on sunlit paths.

Thyridanthrax adults feed at flowers. Gruvel noted that adults appeared at the time of flowering of various plants: *Morelia* (February–March); *Crateva* and herbaceous plants (March–May); *Mitragyna* (August–September); and *Ziziphus* and various shrubs (September–November). *T. abruptus* has often been observed feeding at flowers. Lamborn (1916) and Nash (1970b), have shown that in captivity this species can be kept alive on flowers or on split sugarcane for 3 weeks. However, Gruvel was only able to

keep *T. beckerianus* alive for 1 week when feeding the parasitoids on cotton soaked with a water solution of sugar and vitamins. Thus, *Thyridanthrax* spp. may not have very strict feeding preferences, and difficulty in breeding them may depend less upon nutritional factors than on their general habits (mating, oviposition, etc.).

Data on larval biology and developmental times are available for several *Thyridanthrax* spp. parasitic on *Glossina* (see Heaversedge 1970; Hursey 1970; and Gruvel 1974b). The first larval stage is a 14-segmented planidium, which searches for a host, apparently at random. The inefficiency of such a method is compensated for by the high fecundity of the bombyliid female. The planidia do not seem to be very specific in their selection of a host because *Thyridanthrax* spp. parasitize *Glossina*, calliphorids (e.g. *Rhyncomyia pictifacies*, as shown by MacDonald (1957)), Orthoptera, and even the braconids that are themselves parasites of *Glossina* (Hesse 1956). If, as in the bombyliid genus *Villa*, the planidium stage may last for several weeks, then the chances of its finding a host are very much increased.

Penetration of the larva into a *Glossina* puparium is dependent on a number of factors. Among these is the coincidence of oviposition sites of host and parasite, the nature of the soil at these sites, the depth at which the puparia occur and their density, the number, mobility, and longevity of the planidia, and finally, aspects of climate. Usually a single planidium enters a puparium by gnawing a minute hole with its cephalopharyngeal teeth. If several planidia enter the same puparium only one develops, its second instar occupying a dorsal position in the *Glossina* puparium. This becomes a third-stage larva filling the whole puparium. Puparial formation then takes place. According to Gruvel the puparial stage lasts 11 days in *T. beckerianus* and is very resistant to drying, in contrast to puparia of *Glossina*. In *T. beckerianus*, emerging adults show a sex ratio of 1:1 (Gruvel) as in the case with *T. abruptus*, *T. lugens*, and *T. salutaris*. There are, however, several known instances where significantly more females than males occurred (Heaversedge 1969d).

The size of the adult depends on that of the host puparium from which it developed. Puparia of *G. pallidipes* produce larger *T. abruptus* than do those of *G. morsitans*, which are smaller than *G. pallidipes* (Heaversedge 1968a). Similarly, within the same host species the size of the emerging bombyliid is correlated with that of the host puparium (Fiedler and Kluge 1954; Gruvel 1974b).

There are no data on longevity of adult *Thyridanthrax* in the field. The length of the larval stages varies considerably, depending on the species concerned and the time of year. Heaversedge (1970), studying development of *T. lugens* and *T. salutaris*, found little change during the course of the year — the cycle averaged about 30 days in the laboratory. For *T. abruptus* the average reached 80 days in November and December, but was 30 days in other months. Chorley (1929) suggests the possibility of a diapause of immature stages of *T. abruptus* within the puparium of *G. morsitans*. Nash (1930) observed a 197-day puparial stage before emergence, and Hursey (1970) 270-days with *T. abruptus*. Fiedler et al. (1954) stated that the puparial period could exceed by 90 days that of the host. There is no information as to the causes of such diapause in the immature stages. However, the fact that it occurs is certainly most important, both from the point of view of any accurate determination of percentage parasitism of puparia (since times of collection relative to emergence of host and parasitoid are important), and also in order to establish basic data related to both laboratory breeding and the possibilities of conservation of natural enemies when *Glossina* breeding areas are treated with chemical pesticides. Indeed, if an insecticide is applied only to those areas where *Glossina* adults rest, and if persistence is not excessive, then populations of *Thyridanthrax* might well be able to maintain themselves. There would

be an added advantage here, in that the area of dispersal of these parasitoids is often wider than that treated against tsetse. However, with less selective insecticide applications (e.g. by fixed-wing aircraft or helicopter) a *Thyridanthrax* population may well be eliminated, particularly if organochlorines are used (Fiedler et al. 1954).

Petrorossia

Two species of this genus *P. angustibasalis* Hesse and *P. hesperus tropicalis* Bezzi are referred to by Heaversedge (1968b) as parasitoids of puparia of *G. m. submorsitans* collected in Rhodesia.

Other Parasitoids

Hymenoptera

Chalcididae

Glossina puparia may be parasitized by a number of species of chalcids — see Waterston (1915a, b, 1916, 1917), Hegh (1929), Ferrière (1935a, b), Thompson (1943), Buxton (1955), Jenkins (1964), and Nash (1970b).

Brachymeria amenocles Walker (= *B. varipes* Walker) was reared from puparia of *G. m. submorsitans* and *G. tachinoides* in Ghana (then the Gold Coast) by Simpson (1918). It also occurs in Rhodesia. This species appears not to be restricted to *Glossina*, but records of it are rare. Simpson easily bred several hundred on puparia of *Sarcophaga* sp. and hence considered the possibilities of using it against tsetse. However, its very low incidence in the field makes it improbable that this would prove successful.

Waterston (1917) described *Dirhinus inflexus* from the Gold Coast (Ghana), where Simpson (1918) had discovered it in *G. m. morsitans*. Another species, *D. giffardii* Sylvestri (well-known from Africa in connection with biological control of *Ceratitis capitata* in Hawaii), is recorded from three species of tsetse, (*G. brevipalpis, G. morsitans* (in Ferrière 1935a, b), and *G. palpalis* (in Thompson 1943).

Haltichella edax Waterson appears to be only an incidental parasitoid. Lamborn (1915a, b) reared a single example from *G. morsitans* in Malawi (then Nyasaland).

Stomatoceras micans Waterston and other members of the genus are more often recorded than any of the other chalcids. They are known from Kenya, Tanzania, Rhodesia, Mozambique, and even Nigeria. Their hosts include *G. morsitans, G. tachinoides*, and *G. austeni*, but their incidence is always low. Nash (1933a) obtained a single *S. micans* from 5000 *G. morsitans* puparia. Dias (1961) mentions *S. micans* parasitizing *G. austeni* in Mozambique where *all* parasites together gave only 0.7% parasitism.

Stomatoceras exaratum Waterston has been reared from *G. morsitans* in Malawi and is also found in Rhodesia. *S. schulthessi* is recorded from *G. morsitans* and *G. tachinoides* (Ferrière 1935a, b).

As has been mentioned, parasitism of *Glossina* by chalcidids seems only incidental.

Eupelmidae

Eupelmella tarsata Waterston is known from *G. morsitans* in Malawi (Lamborn 1915b, 1916; Waterston 1916) and in Rhodesia (Chorley 1929). Lamborn, who found only four parasitized puparia in 2000 examined, observed that this species could act as a hyperparasite of *Mutilla glossinae* in *G. morsitans* puparia. In an experiment, nine *Eupelmella* females oviposited in puparia of nine *G. morsitans*, all but one of which contained cocoons of *M. glossinae*. Twenty-two male and fifty-five female *Eupelmella* emerged.

Anastatus viridiceps Waterston has been recorded from *G. morsitans* in northeast Rhodesia and from *G. austeni* in Mozambique (Dias 1963). Baldry (1969) reared an *Anastatus* sp. from *G. palpalis* in northern Nigeria on one occasion. Lloyd (1916),

in Rhodesia, obtained *A. viridiceps* from four puparia of a collection of 900. From each of these emerged adults, mostly females, but Lloyd was unable to breed them.

Diapriidae

Three species of this family have been reared from tsetse: *Trichopria capensis robustior* Silvestri, *T. lewisi* Nixon, and *Abrothropia lloydi* Ferrière. The first is known only from Zululand, South Africa, where it was reared in 1922 from *G. pallidipes*. Its presence there was afterwards confirmed by Fiedler and Kluge (1954). In Kenya, Lewis (1939) recorded parasitism of 45 and 20% by a trichopriid, previously determined as *T. capensis*, in collections of puparia of *G. brevipalpis* and *G. fuscipleuris*, respectively. This same parasite was recorded in Kenya from *G. pallidipes*, and was described by Nixon (1940) as *Trichopria lewisi*.

Although the degree of parasitism given by Lewis is high and *T. capensis* appears to be fairly easy to breed, the effect of these diapriids on tsetse populations is completely unknown. Moreover, other species of *Trichopria* are known to be hyperparasites of tachinids. Therefore, their value is potentially negative.

Bethylidae

Turner and Waterston (1916) observed *Prolaelius glossinae*, which Lamborn (1916) found from *G. morsitans* in Malawi.

Braconidae

A braconid described by Turner (1917) as *Coelalysia glossinophaga* was also reported in Ghanian *G. m. submorsitans* by Simpson (1918).

Ceraphronidae

Conostigmus rodhaini Bequaert is a rare parasite of tsetse. Bequaert (1913) obtained five females and a male from one puparium out of 400 *G. palpalis* collected.

Perilampidae

Perilampus ruficornis F. (= *P. violaceus* F.) is known from *G. morsitans* in Nigeria (Ferrière 1935a, b).

Pteromalidae

Nasonia vitripennis (Walker) (under the name *N. brevicornis*) was mentioned as a parasite of *G. morsitans* by Rouband (1917b), who suggested the possibility of its being used against pest Diptera.

The above Hymenoptera, with few exceptions, have been recorded only once. While detailed information is admittedly lacking as to their role, if any, as control agents of tsetse, it is self-evident that they cannot exert more than a very minor effect because they are only incidental parasitoids of *Glossina* spp. and characteristically attack other hosts. The eulophids *Syntomosphyrum* spp., dealt with in a previous section, have certainly attracted greater attention as *Glossina* parasites than these other Hymenoptera.

Acarina

Although mites are neither insects nor parasitoids in the sense that the term is used here, this is perhaps the most convenient place to mention their occurrence in association with tsetse.

There are a number of records of *Acarina*, either as immatures or adults, from adults of different *Glossina* spp. The exact systematic position of these acarines needs investigation. Fain and Elsen (1972a, b) furnished data on some mites found by them on *Glossina* adults in Zaïre, and they also discussed records by Carpenter (1912, 1913). Additionally, Fain and Elsen furnished original data from Zaïre, recording the following from *Glossina* adults: the sarcoptiformes, *Afrocalviolia glossinarum* Fain and Elsen, and *A. tsetse* Fain and Elsen; species of *Histiostoma* and *Bonomoia*; and trombidiformes of the families Pyomotidae, Pymephoridae, and Eriophyidae, which were found in small numbers, mostly on *G. f. quanzensis*.

Carpenter (1912) recorded 15 cases of parasitism in 9000 adult *G. palpalis* examined in Uganda. Immature stages of *Leptus* were responsible in all cases. Fain and Elsen (1972b) clearly described four new species of this genus as phoretic upon tsetse in Zaïre (*L. carpenteri, L. benzaliensis, L. glossinarum,* and *L. maringensis*).

Macfie (1913) recorded larval *Trombidium* from *G. palpalis* and *G. tachinoides* in northern Nigeria, and Krampitz and Persoons (1966) larval *Erythraeoidea* from *G. fuscipes* in Kenya.

In Upper Volta, Challier (1971b) found hydracarian ectoparasites hooked onto the body and hairs of adult tsetse.

These records notwithstanding, it must be stressed that the occurrence of acarines on tsetse is very infrequent, and that their detrimental effect, if any, is uncertain.

Possibilities of Using Introduced Parasitoids

Quite apart from the parasitoids of *Glossina* spp. which may well be introduced from areas of Africa into others where they do not already occur, there is the possibility of introducing into Africa parasitoids from elsewhere. Since *Glossina* only occurs in Africa, such parasitoids must obviously be those of other hosts. It has been seen from the work already done with *Syntomosphyrum* and to a lesser extent *Mutilla*, that some *Glossina* parasitoids can be successfully bred on alternative hosts. Would it not be possible therefore to find parasitoids of other hosts, probably of dipterous puparia found in the soil, which would successfully attack *Glossina* puparia in the field?

Recently (see, for example, Legner and Olton 1968), there have been widespread searches in different parts of the world for natural enemies of synanthropic flies (e.g. *Musca* and *Stomoxys* spp.) for use in biological control. Mass-breeding techniques have already been developed for some of them, and practical biological control has been attempted on several occasions, notably in California, Hawaii, Mauritius, and New Zealand.

The idea of testing some of these parasitoid species as biological control agents of *Glossina*, together with other possible aspects of biocontrol, was proposed by Simmonds in 1969 (unpublished memorandum). In the present context it would certainly seem to be an approach that should be pursued vigorously since much of the basic research involved in finding, identifying, and mass-breeding a number of these parasitoids has already been carried out. All that remains to be done is to determine whether they will accept *Glossina* puparia as hosts, both in the laboratory and in the field, and whether their biological characteristics (e.g. temperature tolerance, longevity, host-finding ability, reproductive rate, and development time) would enable them to maintain populations in one or more *Glossina* habitats.

Several species of such parasitoids of *Musca*, for example, are known from regions (e.g. East Africa) where *Glossina* exist. Since they have never been recorded as parasitoids of *Glossina* it is reasonable to suppose that they are not suitable as biocontrol agents of tsetse. However, there are a number of other species (e.g. the staphylinid beetle, *Aleochara taeniata* Erichson, and the pteromalid wasp, *Muscidifurax raptor* Girault and Sanders) which *do* in fact occur in (South) Africa. Certain species of *Dirhinus* and *Spalangia* that do *not* occur in Africa, but which are important puparial parasitoids of muscids, also warrant consideration in this regard. There should be no difficulty in obtaining and testing them.

Table 5 lists a few muscid parasitoid species that might be considered as candidates for trial against *Glossina*. Naturally, *Glossina's* peculiar life cycle would render larval or larval-puparial parasitoids of negligible value, since the length of the free larval stage is so very short. Puparial parasitoids on the other hand would be of considerable interest, for in *Glossina* this stage lasts appreciably longer than in

Table 5. Puparial parasitoids of possible interest for trial against *Glossina*.

Parasitoid	Host				Distribution
	Musca domestica	*Stomoxys calcitrans*	*Fannia femoralis*	*Fannia canicularis*	
Staphylinidae					
Aleochara taeniata Erich.	+				
Pteromalidae					
Muscidifurax raptor Gir. & Sand.	+	+	+	+	Widespread (recorded from South Africa)
Pachycrepoideus vindemiae (Rond.)	+			+	Widespread
Dibrachys cavus Walk.	+				Europe, America
Stenomalus muscarum L.	+				Europe
Trichopriidae					
Trichopria spp.	+	+	+	+	Widespread (but some can also be hyperparasites through tachinids)
Spalangiidae					
Spalangia cameroni Perk.	+	+	+		
S. endius Walk.	+	+	+	+	Widespread (including East and South Africa)
S. nigroaenea Curtis	+	+		+	
S. stomoxysiae Gir.	+				Puerto Rico
S. nigra Latreille	+	+			Europe, North America
S. nigripes Curt.	+		+	+	Europe, USSR, N. America
S. simplex Perkins	+				Israel, South Africa
S. longepetiolata Boucek	+	+			East Africa
S. platensis (Brethes)	+	+			Argentina

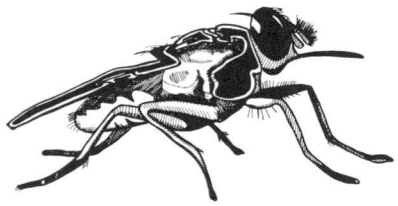
many other Diptera. The actual host selection and behaviour of individual parasitoid species during oviposition, possibly with some species limited only to newly formed puparia, may well be a limiting factor as to the usefulness of some otherwise suitable parasitoids.

What is now required is the setting up of a "testing station" where as many as possible of such "potential" *Glossina* parasites can be tested, in both the laboratory and the field. We need to know whether they can attack and develop in *Glossina* puparia, and under conditions simulating various *Glossina* puparial habitats, so that some idea may be gained as to how they might react if released in the field. Can they, for example, locate *Glossina* puparia in the soil, search adequately for puparia, withstand the temperatures to which they would be exposed, etc.? This would entail a great deal of work, with a regular supply of puparia of several *Glossina* spp., before any field trials could be considered. Because this work would entail routine handling and breeding of a variety of parasitoid species, one of the CIBC stations would be an appropriate location since pertinent expertise is readily available.

Inevitably, some of the parasitoid species tested in this way will not find *Glossina* puparia suitable hosts, and additional work with them would not be called for. Others, which attack *Glossina* puparia readily and find them suitable hosts, might be further studied to determine as far as possible the extent to which they are suitable for seeking out and attacking *Glossina* puparia in their natural habitats. Initially, the necessary work could be done in the laboratory with simulated field conditions. Species found promising on all counts could become the subject of field trials.

Bearing in mind the extensive recent work on using parasitoids of muscid flies in other biocontrol programs, investigations to determine if any of these now well-known parasitoid species might prove

of value against *Glossina* could very easily be undertaken. In addition, further search could be made for parasitoids of other Diptera in habitats similar ecologically to those of some of the *Glossina* target species. Any parasitoids so found could then be subjected to the same screening processes.—F.J. Simmonds, A.M. Jordan, and S.M. Touré.



Pathology and Nematode Parasitism

What can go wrong with a tsetse fly? Disabling effects can result from all of the obvious physical and biological environmental factors that challenge other plants, animals, and microorganisms throughout their lives. Besides obvious effects of insufficient moisture and nutrients, exposure to abnormal temperatures, physical trauma of wounds, effects of chemical pesticides, and inconspicuous errors in development, challenges to life by biological agents (predators, parasites, and pathogens) take a regular toll of tsetse flies and other insects. Accounts of the predators and parasitoids affecting tsetse appear elsewhere in this volume. The microorganisms and nematodes that live at the expense of a tsetse fly without contributing to the life expectancy of the individual are collectively referred to as pathogens herein.[6]

The pathogens include species of microorganisms referable to viruses, bacteria, rickettsiae, fungi, protozoa, and nematodes. Various textbooks and other reference volumes, which can be consulted on the subject of microorganisms associated with insects and their infectious diseases, have been published (Burges and Hussey 1971; Cantwell 1974; Krieg 1961; Paillot 1933; Steinhaus 1946, 1949, 1963; Weiser 1969).

Insects and microorganisms (including nematodes) are found in a variety of associations, which were described by Steinhaus (1954).

1. Insects feeding on substrate previously broken down or changed by the activity of one microbial species; for example, yeasts bringing about fermentation of grapes in nature, thus providing optimal conditions for the developing larvae of *Drosophila*.

2. Free-living microorganisms, especially bacteria and yeasts, serving directly as food for insects; e.g. mosquito and fly larvae feeding directly on bacteria or protozoa present in their environment.

3. Insects and microorganisms existing separately but in a more-or-less common or regular association. Insects acting as carriers or hosts only occasionally, or when specially cultivated microorganisms are ingested as food. Examples of this type of relationship are the fungus-growing ants, and mycetophilous termites and beetles.

4. Insects as hosts to microorganisms present commonly in or on the insects. It is common to find bacteria on soil-inhabiting insects.

5. Insects as hosts to commensal microorganisms found associated with them, in the same manner as *Escherichia coli* is a commensal regularly present in the intestinal tract of humans. Certain species of microorganisms are constantly present as commensals in the alimentary tracts of insects.

6. Insects as vectors of microorganisms pathogenic to animals or to plants. Examples are the mosquito transmission of the agents of malaria and yellow fever, and the

[6] The term "parasite" is more appropriate for nematodes and equally appropriate for fungi and protozoa. It is not used here to avoid confusion with the entomophagous parasitoids of tsetse, to which the term "parasite" usually refers in relevant literature.

tsetse-fly transmission of African sleeping sickness.

7. Insects as hosts to *extra*cellular symbiotes (i.e. mutualists). These are exemplified by: (1) the protozoa living in the gut of termites and enabling the latter to obtain nutriment from ingested wood; and (2) the bacteria that regularly inhabit the specialized areas of the gut of many insects.

8. Insects as hosts to *intra*cellular symbiotes (i.e. mutualists); such as the bacteria and yeast-like microorganisms regularly inhabiting the specialized tissues termed "mycetomes," or other tissues of insects. These microorganisms are apparently necessary for the normal life of the insect, for example *Glossina* spp.

9. Insects as hosts to microorganisms that are semiparasitic upon them, such as fungi of the ascomycete order Laboulbeniales that obligately live on the integument of insects, or those basidiomycetes (genus *Septobasidium*) that parasitize some individual insects in a population while benefiting other individuals of the same species.

10. Insects as definitive hosts of microbial agents to which they are susceptible. In this relationship, the microorganisms cause true diseases in their insect hosts.

An introduction to insect microorganism associations is necessary to appreciate the significance of the occurrence of microorganisms detected within or on different developmental stages of tsetse flies.

Four principal sources include the references to the published reports of microorganisms and nematodes affecting species of *Glossina*: Buxton (1955); Jenkins (1964), a supplement and emendment of the latter by Laird (1971); and Roberts (1976). Nolan's tsetse bibliography in the latter is annotated.

The references cited in this chapter are representative only. They were selected from available bibliographic resources and concentrate upon publications containing experimental evidence to suggest or confirm the intimate association of the microorganism or nematode with its host, and the consequences of that association. Mere mention of the detection of microorganisms or nematodes within or upon a life-history stage of the tsetse fly without evidence of more than an adventitious association, are not considered.

Over many years, efforts have been made to isolate microorganisms and nematodes encountered in the life-history stages of tsetse. These efforts have been relatively minor by comparison with the total investment of labour and other resources for the interruption of disease transmission by the fly. The initiative and dedication of individuals during most of the twentieth century, in locations providing research opportunities, have produced a series of isolated contributions to our knowledge of pathogens. A specific plan for action to implement the routine detection, isolation, and review of pathogens is required to accelerate the effort. The five-stage review scheme implemented by the World Health Organization provides the basis for review of potential biological control agents of tsetse (WHO 1975). Sustained and coordinated efforts to stimulate the detection and isolation of *Glossina* pathogens have yet to be made. However, a service is active and productive for the isolation and identification of pathogens affecting other vectors, through the WHO Collaborating Centres administered by WHO's Division of Vector Biology and Control in Geneva, Switzerland. This existing system could easily be expanded to provide adequate coverage for *Glossina* as well.

Symbiotes

Wallace (1931) reviewed the results of investigations up to and during 1931 on the microorganisms found associated with *Glossina* spp. as commensal or mutualistic symbiotes, and first illustrated by Stuhlmann (1907). The hosts recognized were: *G. palpalis, G. tachinoides,* and *G. brevipalpis.* In adults, the bacteria-like symbiotes are localized in cells of the anterior midgut. The "long bacilli" in the gut

of both wild and laboratory-reared flies were considered by Wallace (1931) to be identical with symbiotes described tentatively as yeasts by Roubaud (1919). In summary, Wallace believed that the then-available evidence was inadequate for identification of the symbiotes as either bacterial or fungal. Attempts to cultivate the symbiotes (= bacteroids) *in vitro* with complex media, and enriched extracts of tsetse gut, either aerobically or anaerobically, were unsuccessful. Hanging-drop preparations in 1% glucose also proved unsatisfactory for culture purposes.

A generalization made by Wallace, and supported by his observations, is that a large proportion of field-caught flies had the gut empty (due to irregular feeding opportunities) in contrast to flies from laboratory colonies. This condition made dissection of the intestinal tract difficult, and consequently increased the incidence of microbial contaminations associated with attempts to isolate gut microbiota *in situ*.

Feeding bacteria to adult tsetse in a live host, or via sheep blood through an animal skin, provided evidence that ingested bacteria can live for up to 30 days in the alimentary tract and can be distinguished from symbiotes and trypanosomes in the lumen. Wallace demonstrated further that flies cannot maintain living microorganisms in the gut when these are simply contaminants of their environment, e.g. surfaces in cages. Thus while *G. palpalis* ingests microorganisms occurring as contaminants of its physical environment, these microorganisms do not survive in the gut of the fly despite repeated exposure.

Bacteriolysins resulting from the activity of symbiotes can account for the commonly demonstrated bacterial sterility of the insect gut. Wallace recognized this possibility, although he felt it "admittedly unlikely, that they (the symbiotic bacteroids) may have a constant adverse action on trypanosomes" and other microorganisms. Wallace, however, speculated that such an effect may be a reason for the low percentage of *Glossina* becoming "infected" (and, in due course, effective vectors) when fed on an animal parasitized by trypanosomes. Experiments by Wallace in which bacteria were experimentally introduced into flies appeared to demonstrate inhibition of trypanosome activity. One question does arise, however: If bacteriocidins are active against bacteria introduced into flies from the environment, e.g. surface contaminants, why do bacteria introduced with blood meals remain alive up to 30 days? Three out of five experiments, each involving 50 laboratory-reared flies, led to the disappearance of bacteria within 10 days. The experiments were believed by Wallace to involve too few flies, considering the variety of factors influencing the success of laboratory maintenance.

Wallace (1931) conducted studies with *G. palpalis* that, in contrast to *G. morsitans*, appears to be less affected in laboratory culture by bacterial contamination. Rodhain et al. (1913) were reported by Wallace to specify that the apparatus used for membrane-feeding *G. morsitans* must be free from contaminating bacteria, which are known to multiply in the gut and cause the death of the flies.

Sensitivity of *G. morsitans* to microbial contamination was reported by Wetzel and Bauer (1975). According to these researchers an alternative to aseptic (gnotobiotic) feeding of flies is the use of oxytetracycline at 25 ppm in defibrinated blood. The antibiotic is necessary to prevent a fatal bacterial infection characterized by melanized abdomens. The unacceptable consequence of the antibiotic therapy was a reduction in fly fecundity. Follicular development is inhibited if the follicle is less than one-fourth "mature size," whereas, larger follicles or intrauterine larvae are unaffected. If the oxytetracycline is administered up to 7 days after emergence a 90% reduction in fecundity results. Administration to flies 20–30 days after emergence reduces fecundity by 50%.

Adult female *G. morsitans* have been similarly subjected, via injected host rabbits, to treatments with streptomycin sul-

fate, penicillin G, chloramphenicol, and polymyxin. This resulted in a subsequent loss of fecundity that was correlated with loss of symbiotes (Hill et al. 1973). These investigators also subjected larvae (third instar) to a range of antibiotic powders at pupation sites. Although the results were not conclusive, reduction of symbiotes was claimed to result from absorption of antibiotics through the larval integument.

Pertinent to the experiences of Hill et al. (1973) and Wetzel and Bauer (1975) is the description of bacteria from the milk gland of *G. m. morsitans*. These are considered to be symbiotic microorganisms (Ma and Denlinger 1974). Similar observations have been made for *G. austeni, G. longipalpis,* and *G. pallidipes*. Ma and Denlinger stated that on the basis of morphological differences between the described milk gland bacteria, and the early observations cited above (Wallace 1931) "...it is not possible to reject the hypothesis that the fimbriated bacteria represent the free-living form of the intestinal bacteroids."

In the opinion of Huebner and Davey (1974), the bacteroids demonstrated in the ovaries of *G. austeni* provide evidence of transovarian transmission of symbiotes. The investigation suggested "...an alternative or parallel mechanism" for transmission of bacteroid symbiotes "...from one generation to the next..." without "...the externalization of the bacteroids into the lumen of the milk gland and their subsequent penetration into the cells of the gut of the larva."

The role of the symbiotes, which are generally accepted as bacteria-like, appears to be mutualistic. Their contribution to each of the life-history stages of tsetse can be extrapolated from results of investigations with mutualistic symbiotes in other insects. Nogge (1974) attempted to compensate for the loss of symbiotes by giving vitamin supplements, following the cleansing of flies of their symbiotes, either by means of oral treatments or injections of lysozyme.

Investigations dealing directly with symbiotes, and the influence on tsetse of microorganisms in the flies' environment, suggest the following conclusions:

(1) microorganisms can be introduced into the gut of adult tsetse via living hosts or membrane feeding;

(2) species of *Glossina* differ in their sensitivity to microorganisms contaminating blood meals from living hosts or membranes;

(3) microorganisms introduced into the fly during blood feeding can live in the gut for 10–30 days;

(4) bacteria from a contaminated physical environment cannot be successfully introduced to the gut of tsetse flies;

(5) the tsetse gut is generally sterile except for the presence of intracellular bacteria-like symbiotes, and organisms ingested with blood meals; and

(6) antibiotic or lysozyme treatments adversely affect the fecundity and the intracellular symbiotes of individual flies.

Bacteria and Spirochaetes

Circumstantial evidence from Wallace (1931) and Wetzel and Bauer (1975) (see Symbiotes) indicates a greater susceptibility of *G. morsitans* than *G. palpalis* to bacterial contaminants[7] of mass-rearing apparatus. Although precise information on the microbial species investigated is not available to confirm this generalization, the reports of Roubaud and Treillard (1935, 1936) provide supporting evidence. A coccobacillus isolated from puparia of *G. morsitans* and described as *Bacterium mathisi* by Roubaud and Treillard (1935) was shown to be lethal for adult tsetse following apparent ingestion during feeding. Exposure took place when the hair and skin of guinea pigs were contaminated with the bacterial suspension. Further efforts to

[7] Many species-identifications of such saprophytes were published by Nobre and Santos (1970) and Oliveira and Nobre (1970). The genera concerned are footnoted on p. 146 (ed).

isolate *G. mathisi* from *G. palpalis* or to demonstrate its lethality for *G. palpalis* failed. Wallace (1931) had already demonstrated that bacteria may only be ingested by *G. palpalis* when incorporated in the blood-feeding system.

The technical inadequacy of investigations with *B. mathisi* may be only one element contributing to the practical failure of this microorganism. In addition to the failure with *G. palpalis*, inconclusive evidence of the pathogenicity of *B. mathisi* resulted from its preliminary screening for activity via ingestion by *Musca domestica, Culex pipiens, Aedes (Stegomyia)* sp., and *Aedes aegypti*. Isolates of *B. mathisi* were not maintained for future study.

Wallace (1931) identified as *Bacterium prodigiosus* a microorganism pathogenic when fed to *G. palpalis* in blood. The microorganism used, which is now believed to have been *Serratia marcescens*, was "... isolated from a brown iridescent scum on the surface of water in the Botanic Gardens, Entebbe (Uganda)." This is a superb example of serendipity in insect pathology, recognized and recorded by Wallace the microbiologist. Isolates were not retained for pathogen investigations, but Wallace did mention that individual flies became "fragile" when infected with the bacteria.

The infrequent observations, detection, and isolation of bacteria from *Glossina* are due to individual scientists who, without sustained support for their initiative, recorded the association of bacteria with tsetse life-history stages. In recent years, Rogers (1973) described bacteria-like microorganisms in the spermathecae of uninseminated female *G. pallidipes* from field populations in Uganda, whereas Gruvel (1970a) has reported *Bacillus* sp. from puparia of *G. tachinoides* in Chad.

The rediscovery and isolation of Gruvel's *Bacillus* sp. could provide the initiative for development and review of a microbial agent in the WHO five-stage system (p. 160–164). Success in the evaluation of species and strains of *Bacillus* for vector control is exemplified by the activities of Singer (1973). The importance of having a formal procedure for detection, isolation, identification, and development of an invertebrate pathogen provides the opportunity for developing an industrial incentive for its mass propagation.

Singer (1973) and Briggs (1960) both investigated bacteria pathogenic for certain groups of Diptera. As bacterial pathogens of tsetse become available to be considered for development, investigators must provide opportunities for screening additional dipteran bacterial agents (such as those isolated by Singer 1973 and Briggs 1960) or their metabolic products against tsetse. In this connection, one of the saprophytes listed by Nobre and Santos (1970) from *G. morsitans* and from contaminated blood used in their artificial feeding was the well-known candidate microbial control agent, *Bacillus sphaericus*.

Spirochaeta glossinae Novy and Knapp, from the gut of *G. palpalis*, is listed under the emended name of *Borrelia glossinae* in Jenkins (1964).

Fungi

The reports of fungi associated with tsetse, published up to the report of Vey (1971), are consistent in *not* providing identification of the fungi nor evidence of their pathogenicity. However, Oliveira and Nobre (1970) isolated fungi of four genera (*Candida, Cryptococcus, Torulopsis*, and *Rhodotorula*) from the integument of normal *G. morsitans* adults fed on guinea pigs in the Lisbon colony.

Macfie (1916) reported an undetermined fungus from *G. palpalis*, in Ghana. The mycosis affected adults, hyphae being detected within the abdomen. *G. palpalis* adults were reported infected by an unidentified phycomycete in Tanzania, but investigations beyond the recognition of this mycosis were not continued due in part to pessimism concerning its utility (Swynnerton 1936). Adult *G. morsitans* collected in Tanzania during comprehensive ecological investigations by Nash

Sporocysts and free sporozoites of Hepatozoon pettiti from oocyst in haemocoel of adult G. palpalis caught at Akerri, Nigeria, late July 1975 (M. Dickson).

(1933a), proved to be affected by an unidentified phycomycete. He concluded that the mycoses are less important as a population-regulating factor, than other more substantial causes of tsetse mortality in rainy periods. Adult females of *G. morsitans* were found to be subject to an unidentified fungus in Nigeria, where approximately one-third of field-collected females were affected in the wet season (Lester 1934). Lester also reported an unidentified fungus affecting some 10% of the field-collected *G. tachinoides* females.

An "unidentified phycomycete" was also reported from the abdomen of adult *G. brevipalpis*, captured in the Somali Republic by Moggridge (1936), who noted that the organism resembled one causing mycoses in Tanzania.

Vey (1971) recently showed that two mycoses affecting puparia of *G. fusca congolensis* in the Central African Republic are due to *Absidia repens* and *Penicillium lilacinum*. Both fungi being commonly isolated from soil, Vey considered them to be normally present in natural larviposition and puparia-formation sites. Infectivity studies confirmed that Vey's fungi are primary pathogens and not merely contaminants of wounds.

Protozoa

Biologists engaged in tsetse research are primarily occupied with the disease-causing protozoa (*Trypanosoma* spp.) transmitted by *Glossina*; consequently, the fact that only a few protozoan parasites have been detected is surprising.

The protozoan order Microporida has been recorded from a wide range of arthropods, especially insects, whenever sus-

tained efforts have been made to detect insect pathogens. These protozoa are particularly evident among insect colonies. Although microsporidans have not been described from tsetse life-history stages, it is submitted that they are likely to be found in these insects.

Thiroux (1910) described *Haemogregarina pettiti* from crocodiles. Of the subsequent records, most concern the widespread *Crocodilus niloticus*, but Theiler (1930) reported what appeared to be two different species of these parasites from crocodiles of two other genera in Liberia. Chatton and Roubaud (1913) recorded the presence of *Hepatozoon*-like haemogregarines in the haemocoel of four of 465 "wild" *Glossina palpalis* from Dahomey (The Peoples' Republic of Benin), and Macfie (1916) made a similar observation from the same insect at Accra, Gold Coast (Ghana), about 3% of his flies being parasitized. Hoare (1932a, b) elucidated the life-history of Thiroux' organism, and transferred it to *Hepatozoon*.

Hoare (1932a) provided detailed information on the stages of *Hepatozoon pettiti* (Thiroux) in the haemocoel of Ugandan *G. palpalis* (as present in one of a number of individuals fed on an infected *C. niloticus* 20 days before). Oocysts, sporocysts, and sporozoites were present. In 1975, the tsetse stages of a haemogregarine comparing in every way with those illustrated by Hoare (1932a) were discovered by K. Riordan in a "wild" *G. palpalis* captured at Akerri, Nigeria. The fly was the first in which such an infection was noted out of some 10 000–12 000 examples carefully dissected in surveys conducted for the Nigerian Institute of Trypanosomiasis Research (NITR) over a period of several years. Figures, herein, illustrate the oocyst, sporocysts, and sporozoites of the Nigerian parasite, which is considered referable to *H. pettiti*.

Contrary to what is commonly regarded as the normal behaviour of tsetse trypanosomes, one of these flagellates was observed in Liberian *G. palpalis* and *G. pallicera* only in the midgut, hindgut, and haemocoel (Foster 1963, 1964). Its incidence was approximately 4% in the former fly, and 12% in the latter. It was not detected in *G. fusca* or *G. nigrofusca*. The organism's identity was not established, but it exhibited similarities to the tsetse-borne *Trypanosoma grayi* (Novy and Knapp 1906), the blood stages of which occur in crocodiles.

Mshelbwala (1972), working with *Trypanosoma brucei* Plimmer and Bradford, (strain 36/20) at Vom, Nigeria, established infections in *G. tachinoides, G. morsitans*, and *G. palpalis*. All these flies emerged in the laboratory from field-collected puparia. Subsequently, 30 of 890 *G. tachinoides*, 9 of 331 *G. morsitans*, and 1 of 64 *G. palpalis* proved to have both midgut-form tryptomastigotes[8] and salivary-gland-form metacyclic trypanosomes in the haemocoel of the thorax and legs. Some of the flies were demonstrated to be infective to mice, but adverse consequences (if any) to the tsetse themselves were not reported. Otieno (1973) subsequently showed delayed morphogenesis in *T. brucei* from the haemolymph of 2 of 36 parasitized *G. morsitans*, declaring "that the two environments, namely the haemocoel and the gut of the tsetse fly, have different survival values for this organism." He further submitted that "the invasion of the salivary glands through the haemolymph is a more reasonable hypothesis than the hitherto accepted theory," a view that Baker et al. (1975) have since supported. Finally, in a synopsis of *in vitro* growth of trypanosomes in tsetse tissue cultures, Cunningham (1975) pointed out that "comparable tissues from nonhaematophagous *Sarcophaga*, a dipteran closely related to *Glossina* (proved) capable of supporting the growth of (tsetse) trypanosomes." This affinity might well have interesting implications for studies of entomopathogens of *Glossina*.

Although none of the above reports furnish evidence of pathogenicity of these haemocoelic flagellates and sporozoans for tsetse, the term "infection" is used by the authors in the sense of the organisms de-

veloping within and at the expense of their vectors. It is thought probable that any protozoan that penetrates the gut wall into the haemocoel, and subsequently reenters the upper part of the digestive tract prior to being injected into the vertebrate host, causes some measure of harm to the invertebrate digestive tract.

Investigators handling these insects should always be alert to the possibility of encountering not only trypanosomes and haemogregarines, but also such widely occurring entomopathogenic protozoa as Microsporida. Although the use of protozoa as biocontrol agents is so far only modestly developed, it should be noted that Henry (1975) and his co-workers are making encouraging progress in large-scale field trials of a microsporidan against rangeland grasshoppers in Montana, USA (see p. 184). Allowance for a role for protozoan infection is thus a factor to be considered in the construction of future life tables for *Glossina* spp.

Viruses and Rickettsiae

Virus-like particles have been described from the cytoplasm of salivary glands of adult *G. m. morsitans* (Jenni 1973) and from the nuclei of midgut epithelial cells of *G. f. fuscipes* (Jenni and Steiger 1974). Flies of both species were reared at the Swiss Tropical Institute, Basel, Switzerland, from puparia from Tanzania (*G. m. morsitans*) and Uganda (*G. f. fuscipes*). The salivary-gland particles were also identified at the East African Trypanosomiasis Research Organization (EATRO) laboratories at Tororo, Uganda, from *G. m. morsitans* reared from Tanzanian puparia. Jenni and Steiger (1974) found virus-like particles in "practically all nuclei of the midgut epithelium," the masses increasing in size with the age of the flies (between days 20 and 30). After examining "weak" adults, Jenni and Steiger speculated that the presence of the particles might be related "to the high mortality of young *G. fuscipes* (within 10 days after emergence) and to the low emergence rate observed." Transovarial transmission was suggested by Pell and Southern (1975a) and later demonstrated (Pell and Southern 1975b).

Intracellular rickettsia-like organisms have been described in: midgut epithelium; cells associated with the fat-body; developing oocytes; and in association with muscle cells in adults of *G. brevipalpis, G. fuscipes, G. morsitans,* and *G. pallidipes* (Reinhardt et al. 1972; Pinnock and Hess 1974). The flies were from Uganda and Tanzania (courtesy of EATRO) and the Tsetse Research Laboratory (University of Bristol). These rickettsia-like organisms were present in a minority of the insects or tissues examined. However, their occurrence is suggested to be associated with "lytic" zones surrounding them, and host cell "disruption and degeneration" (Pinnock and Hess 1974). Working with Bristol *G. morsitans*, Southwood et al. (1975) established *in vitro* cultures of a midgut rickettsia-like microorganism, the pleomorphism of which suggested that the bacteroids described from ovaries of *G. austeni* by Huebner and Davey (1974) might be one and the same organism. Most importantly, Southwood et al. showed that the organism was sensitive to antibiotics and that tsetse are dependent upon it, opening the possibility of aposymbiotic control of *Glossina* for example, "administering" antibiotics to peridomestic tsetse populations via pigs).

Nematodes

Leiper (1910) described a nematode found by A. Gray in the body cavity of an adult *G. palpalis* (= *G. f. fuscipes*) in Uganda. This immature mermithid, which was about 75 mm (3 inches) long, was

[8] While this was being prepared, Evans and Ellis (1975) reported the penetration of midgut cells of *G. m. morsitans* by *T. brucei*.

probably the first nematode ever recorded as parasitizing a tsetse fly. Minchin (Lloyd 1912a, b) found a single mermithid in an adult *G. palpalis* in Uganda, whereas Lloyd reported that *G. morsitans* is a host for these worms. Carpenter (1912), again in Uganda, found "a minute larval nematode" in the gut of a laboratory-bred specimen of *G. palpalis*. No identification was given, but his drawing shows the worm to resemble a young larval mermithid.

Carpenter (1913) later reported finding four parasitized flies of the same species, each containing a single worm. All these nematodes were located in the abdominal cavities of the flies, which were not believed to be inconvenienced by the presence of the worms (one of which was about 50 mm (2 inches) long). Carpenter did not identify the worms beyond calling them adult helminths, nor did he state the season of capture of the infected flies. Altogether about 1000 flies were dissected.

Lloyd (1912b), in Northern Rhodesia (Zambia), encountered five mermithid nematodes in *G. morsitans* — probably *G. m. morsitans* Westwood 1850 (see Machado 1970). The first specimen was found in a tube in which tsetse (and possibly other insects?) had been stored. The other four were found during the dissection of about 300 flies. One worm was in a female fly, which had been in captivity for 10 days, one was in a male fly, and the remaining two were in a single male captured 19 days previously. All the nematodes were found during February and early March, near the end of the wet season.

Four parasitized specimens of *G. morsitans* — *G. m. centralis* Machado 1970 — were reported by Rodhain et al. (1913) in Katanga (now part of Zaïre). Two of the flies were captured in the field; the others were laboratory-bred specimens fed on goats. Each fly contained a single worm, defined as a larval Mermithidae. The two longest examples measured 28 and 40 mm.

Mermithid nematodes were also found in adult *G. morsitans* in Tanganyika (now part of Tanzania) by Thomson (1947). It is not possible from the available data to decide whether the host was *G. m. morsitans* Westwood or *G. m. centralis* Machado. Three worms were discovered, in three presumably adult tsetse, during dissection of "some 1500" flies caught in the wet season. Their average length was 79 mm and all were located in the host's abdominal cavity. Thomson considered that the presence of such large nematodes would have seriously affected the flies.

The most recent report from Uganda is that of Moloo (1972). During dissection of 5000 examples of *G. fuscipes, G. pallidipes,* and *G. brevipalpis,* one male of the last species was found to harbour two nematodes. The worms, described as members of the Mermithidae, were in the fly's abdominal haemocoel. One worm measured 86 mm in length. Moloo stated that the parasites probably affected the fly's feeding behaviour.

The first record of mermithids in West African tsetse flies was that of Foster (1963), who found 15 infected flies out of 4001 specimens of *G. palpalis* dissected in Liberia. One fly, a male, contained two worms. The others, nine males and five females, each contained one worm. All the infected flies were caught during wet seasons. The nematodes were all found in the abdominal cavity of the host and ranged in size from 32 to 95 mm. Challier (1971b) reported a mermithid (under the group designation "*Agamomermis*") from *G. tachinoides* in Upper Volta.

Mermithids were found in adult tsetse of three species, *G. palpalis, G. longipalpis,* and *G. m. submorsitans,* collected from four areas in Nigeria. The northernmost area, Gamagira, is situated in the North Guinea Savannah (Keay 1949). The other three, Guni, Akerri, and Abuja are in the South Guinea Savannah. After capture, the flies were kept in small net-covered cages and fed daily on chickens. Once weekly, all remaining live flies were sent to the laboratory (NITR, Kaduna) for dissection. Male and female tsetse were dispatched in separate containers.

Table 6. Incidence of parasitization by mermithids of *Glossina* in Nigeria (number of flies parasitized/number examined). Original data, K. Riordan.

	Guni				Akerri		Gamagira		Abuja
	longipalpis		*palpalis*		*palpalis*		*morsitans*		*palpalis*
	male	female	male	female	male	female	male	female	female
1972									
April	—	—	—	—	—	—	0 (80)	—	—
May	0 (21)	0 (44)	[a]4 (70)	[a]4 (101)	—	—	0 (154)	0 (63)	—
June	6 (264)	0 (46)	4 (110)	1 (218)	0 (21)	0 (222)	0 (140)	0 (249)	1 (176)
July	0 (155)	0 (157)	—	0 (103)	—	1 (331)	—	0 (363)	0 (64)
August	—	—	—	—	—	0 (265)	—	1 (193)	0 (99)
September	1 (138)	—	0 (121)	—	—	1 (387)	—	1 (286)	0 (85)
October	2 (125)	—	1 (161)	0 (260)	—	0 (359)	—	0 (252)	—
November	0 (83)	—	0 (54)	0 (139)	—	0 (159)	—	0 (271)	—
December	0 (141)	—	?1 (167)	0 (105)	—	0 (38)	—	0 (229)	—
1973									
January	—	—	—	0 (37)	—	0 (119)	—	0 (168)	—
February	0 (145)	0 (10)	0 (145)	0 (62)	—	0 (91)	—	0 (118)	—
March	0 (58)	—	0 (20)	0 (25)	—	0 (93)	—	0 (81)	—
April	0 (397)	0 (5)	0 (153)	0 (41)	—	0 (265)	—	0 (50)	—
May	0 (63)	—	0 (121)	0 (84)	0 (18)	0 (135)	—	0 (24)	—
June	2 (164)	—	4 (100)	1 (117)	0 (34)	0 (152)	—	0 (40)	—
July	1 (123)	—	6 (171)	0 (110)	—	0 (107)	—	0 (147)	—
August	0 (46)	—	—	—	—	—	—	—	—
Totals	12 (1923)	0 (262)	20 (1393)	6 (1402)	0 (73)	2 (2723)	0 (374)	2 (2534)	1 (424)
			38 (4980)				5 (6128)		
					43 (11108)				

[a] Includes in each case one postparasitic mermithid larva.

For female flies the dissection procedure was designed to reveal the presence of trypanosomes and to permit assessment of physiological (reproductive) age by the method of Saunders (1960c) and Challier (1965). This involved full dissection of the abdomen. Any nematodes present were thus immediately seen. For males, the procedure was designed solely to reveal nematodes by abdominal dissection. A few heads and thoraxes were also dissected, without any nematodes ever being located.

From April 1972 to August 1973, 11 108 adults of the three species of tsetse were dissected. Mermithids were found in 41 flies, and two more were found free in the containers used to transport the flies. A total of 51 immature nematodes was detected. The geographical and seasonal incidences of parasitization are given in Table 6. Guni was a locality of particularly high incidence, both species of tsetse occurring there being affected. The wet season in the Guinea Savannah zone lasts from approximately April to October, starting later and ending earlier in the north than in the south. All infected flies (with one possible exception) were caught during wet months. The possible exception — a *G. palpalis* from Guni, December 1972 (worm No. 20 in Table 7) — had died and started to decompose before being dissected. What appeared to be the remains of a nematode were found, but identification was not certain. All the other worms were intact and usually alive. At Guni, male *G. palpalis* were more often parasitized than females. Thirty-five flies (26 males and 9 females) contained one worm each, four flies (three males and one female) contained two nematodes each, and two flies (both male) contained three nemotodes each. There is no obvious or constant difference between the ranges of size of the worms found in male and female flies nor between those from the three tsetse spec-

Table 7. Nematodes found in male tsetse flies from Guni (original data K. Riordan).

Worm No.	Host fly: Date caught	Host fly: Species	Nematode: Length (mm)	Nematode: Diameter (mm)
1	28 May 1972	*palpalis*	30	—
2			55	0.21
3			50	0.20
4[a]			90	—
5	5 June 1972	*palpalis*	57	0.32
6	5 June 1972	*longipalpis*	25	0.15
7			39	0.15
8	6 June 1972	*longipalpis*	40	—
9			25	—
10	11 June 1972	*palpalis*	63	0.30
11			38	0.27
12			78	0.32
13	11 June 1972	*longipalpis*	57	0.22
14			50	—
15	12 Sept 1972	*longipalpis*	8	0.10
16	8 Oct 1972	*longipalpis*	25	0.22
17	16 Oct 1972	*palpalis*	84	0.20
18	29 Oct 1972	*longipalpis*	56	0.37
19			6	0.16
20[b]	Dec 1972	*palpalis*	—	—
21	16 June 1973	*palpalis*	25	0.16
22			19	0.16
23	16 June 1973	*longipalpis*	25	—
24	24 June 1973	*palpalis*	63	0.22
25			38	0.22
26			31	0.20
27			—	0.20
28	29 June 1973	*palpalis*	36	—
29			11	—
30	4 July 1973	*palpalis*	46	0.16
31	5 July 1973	*longipalpis*	57	0.20
32	10 July 1973	*palpalis*	62	0.22
33			41	0.20
34			41	0.16
35	15 July 1973	*palpalis*	27	—
36	20 July 1973	*palpalis*	48	0.32
37	31 July 1973	*palpalis*	46	0.16
38			53	0.22
39			49	0.16

[a] Postparasitic larva found free in container.
[b] Doubtful record based on possible relic in decomposed fly.

ies (Table 7 and 8). In two instances where flies contained two worms each, the worms in the same host were markedly different in size (No. 18 and 19 in one fly, and No. 41 and 42 in the other).

The mermithids varied greatly in size and included some very short specimens. Generally, however, they were of the same order of size as those described from tsetse by previous authors. For infected female flies (Table 6) there was no correlation between fly age, assessed by evidence of the

Table 8. Nematodes found in female tsetse flies from Guni, Akerri, Abuja, and Gamagira (original data K. Riordan).

Worm No.	Host fly: Date caught	Locality	Species	Age (days)	Nematode: Length (mm)	Diameter (mm)
40	22 May 1972	Guni	*palpalis*	20	70	—
41	23 May 1972	Guni	*palpalis*	10	5	—
42	23 May 1972	Guni	*palpalis*	10	30	—
43	30 May 1972	Guni	*palpalis*	10	25	—
44[a]	30 May 1972	Guni	*palpalis*	—	70	—
45	5 June 1972	Guni	*palpalis*	50	27	0.16
46	25 June 1972	Guni	*palpalis*	10	108	0.32
47	1 Aug 1972	Akerri	*palpalis*	50	46	0.22
48	19 Sept 1972	Akerri	*palpalis*	55	79	0.20
49	25 June 1972	Abuja	*palpalis*	30	—	—
50	5 Aug 1972	Gamagira	*morsitans*	40	6	0.11
51	15 Sept 1972	Gamagira	*morsitans*	30	63	0.16

[a] Postparasitic worm found free in container.

number of ovulations, and the size of the contained nematode(s). The longest worm (No. 46) was in a young fly; the next longest (No. 48) was in a much older fly. Three old flies contained short worms (No. 45, 47, and 50). All the nematodes found inside flies were loosely intertwined with the internal abdominal organs. There was never any attachment between a worm and any of the host's organs.

The two worms (No. 4 and 44, Tables 7 and 8) found free in the containers were presumably postparasitic larvae that had emerged from flies being transported. In the case of worm No. 4 a recently dead tsetse was found in the container. This fly had a large hole in its ventral abdominal body wall, through which the nematode had obviously emerged.

It was not possible to identify parasitized *Glossina* by external examination, nor, on dissection, could any abnormality of the internal organs of the host be seen. Of the 10 infected female tsetse only two had developing progeny *in utero*. One of these had an egg in the uterus and showed evidence of four previous ovulations. The other contained a nearly full-grown larva and had ovulated three times. Of the remaining eight flies, three had not ovulated and five had ovulated from one to five times.

Specimens were sent to Dr W.R. Nickle of the Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland. He confirmed that they were mermithids, but could not provide a more complete identification, due to the absence of adult forms. These are the first reports of mermithids as tsetse parasites in Nigeria. The absence of earlier records is somewhat surprising, because three tsetse species, from four widely separated localities, were found to be infected. Larval nematodes from Nigerian *Glossina* more recently given to Nickle by B.A. Matanmi have been reported by the former (personal communiation to ed) to resemble terrestrial mermithids of the *Mermis-Hexamermis* type, which often take 60–90 days to become adults in the soil.

The stage and site at which tsetse become infected by nematodes remain unclear. Specimens were found in laboratory-bred flies by Carpenter (1912) and Rodhain et al. (1913). In explaining such cases, Foster (1963) suggested that nematodes were present in the moist earth over which puparia and flies may have been kept, and that they entered newly emerged flies. Moloo (1972) proposed a similar mode of entry. Most cases of infection, however, have involved wild tsetse. Foster

(1963) suggested that it is the resting adult flies that are usually penetrated by mermithids that have wriggled up vegetation. In support of this, he pointed out the seasonal nature of infestation, arguing that nematodes are absent from dry-season tsetse resting-sites.

The lack of correlation between fly age and nematode size could indicate that the mermithids enter resting flies on vegetation, since flies of any age could be involved. The rate of development of individual mermithids (in other insects) is known to vary greatly. Therefore, infection of tsetse during the larval or puparial[9] stages cannot be ruled out. The occurrence of multiple infections in single flies is similarly inconclusive. Obviously, the infection of a tsetse (whether immature or mature) can occur just once, or more than once.

Nickle (1972) described several types of life cycles known to occur among the Mermithidae. In the case of *Perutilimermis culicis*, the young nematode penetrates an early instar mosquito larva, remaining dormant throughout the rest of the host's juvenile development. Only when the adult mosquito has emerged does the nematode start to grow. There seems no reason why a similar cycle cannot apply in the case of mermithids parasitizing tsetse. The fact that in the present study male flies were seen to be more often parasitized than females, suggests that infection usually occurs during adult life. It is unlikely that larvae destined to be male or female tsetse are deposited in different sites or that they have different protective mechanisms. Adult males are in fact more active than females and visit more resting sites. Thus, they have a greater chance of encountering preparasitic nematodes.

The data indicate that a tsetse is not seriously inconvenienced by the internal presence of a mermithid. The volume of even the largest worm detected would have been very considerably less than that of a large blood meal or of a well-developed tsetse larva. Parasite size alone does not seem to be important. According to Nickle (1972), mermithids are important in the natural control of populations of a variety of insects. It is unlikely that this is the case for tsetse. The apparent low incidence of this type of parasitism led Moloo (1972) to suggest that mermithid infections of tsetse are accidental. Whether or not this is usually so, it remains to be established whether or not the situation at Guni, as described above, involves a specific parasite of *Glossina*.

Evidence of natural maladies among *Glossina* spp. have rarely been reported. Most reports deal with the incidence of disease in adults, an important exception concerning the fungi isolated from puparia by Vey (1971). Adults are the life-history stage most exposed to a broad variety of environmental challenges, whether in the field or the laboratory. Larvae *in utero* are of course exposed along with the adults, but direct attacks on them by infectious pathogens appear possible only through exploitation of the intimate adult-larval contact. The larvae benefit from the combination of a protected habitat in the abdomen of the female, and her behaviour, which has great survival value. Puparia, although obviously exposed to biological agents in the period of quiescence following the brief phase of larval activity, have been the source of only a solitary confirmed pathogen isolation. The difficulty of locating larvae and puparia, by comparison with the relatively easily trapped adults, can be correlated directly with the frequency of pathogen isolation from tsetse. To date the most conspicuous pathogens (the nematodes) are the ones that have been most often recorded from the tsetse adults, which are the most conspicuous and accessible life-history stage.

Before considering seriously the use of microbiological agents for tsetse control, investigators must understand the nature and the incidence of pathologies affecting tsetse. It is presumptuous to anticipate exploitation of pathogens for vector control,

[9] Since this was written George O. Poinar, Jr. has recorded the nematode invasion of a puparium of *G. morsitans* via the respiratory (polypneustic) lobes (p. 90, 91).

before their effect on the life history of the fly is understood. The effect of the pathogen on the ability of the fly to serve successfully as a vector must be ascertained. First and foremost, as a basis for research that will clarify the feasibility of using mass-produced pathogens against *Glossina*, we must have the assurance of an adequate range of candidate biocontrol agents, which is only attainable through well-organized surveys throughout Africa's tsetse belt. — J.D. Briggs, K. Riordan, S.M. Touré, and R.A. Nolan.

Experiments with Organisms from Hosts Other than *Glossina*

Associations of pathogens with tsetse flies in nature and under laboratory conditions have already been detailed. This synopsis supports IDRC (1974) in emphasizing how little information is available on the identification, occurrence, and importance of these organisms as biocontrol agents.

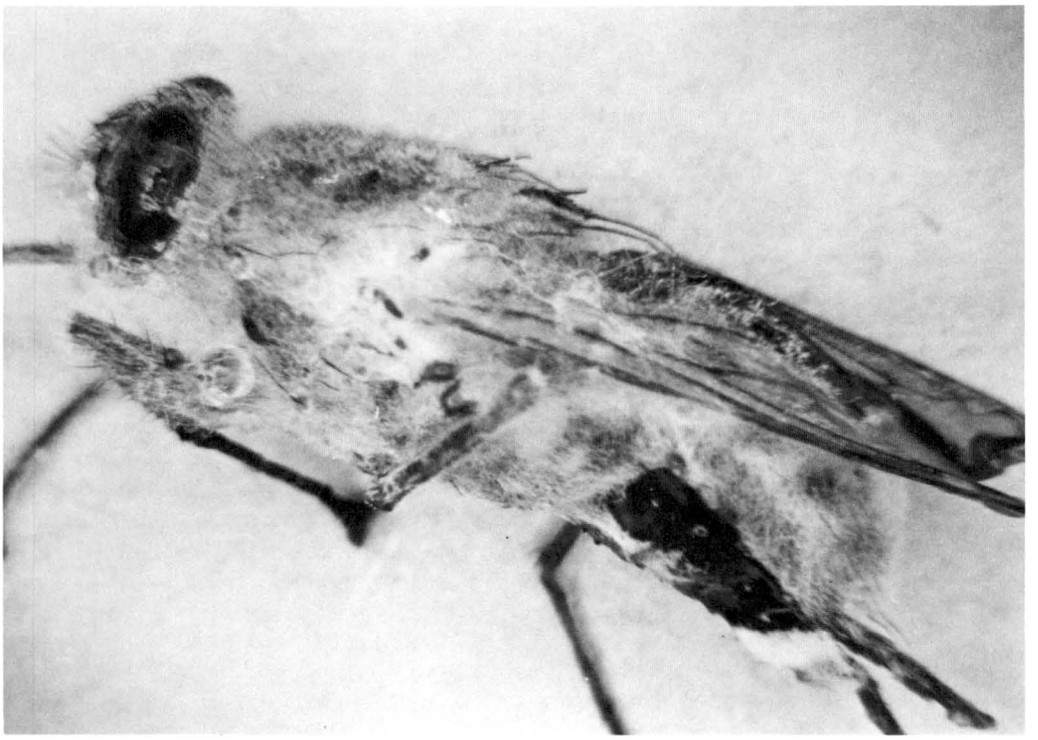
Also evident from this discussion is the past lack of effort to test other pathogens against various stages of *Glossina* species. Yet, this would appear to be a logical step for initial recognition of candidate biocontrol agents against tsetse. An investigation was conducted in the Laboratory of Entomology, Amsterdam, in the summer of 1976, to test the susceptibility of one of the most efficient vectors of human trypanosomiases to some common insect pathogens. It is hoped that these preliminary studies will stimulate others to search further for natural pathogens as well as continue infectivity tests with pathogens isolated from other diseased insects.

Tests were conducted against puparia and adults of *G. m. morsitans* colonized at the Department of Entomology, University of Amsterdam. Cultures of the insect pathogens used in this investigation were provided by G. M. Thomas from the culture collection of the Insect Pathology Diagnostic Laboratory at the University of California, Berkeley. The fungi used were: (1) *Nomurea rileyi* (Farlow); (2) *Hirsutella* sp., (3) *Spicaria farinosa* (Dichs & Fr.); (4) *Beauveria bassiana* (Balsamo); and (5) *Metarrhizium anisopliae* (Metchnikoff). The bacteria tested were *Serratia marcescens* Bizio and *Bacillus thuringiensis* var. *thuringiensis* Berliner; whereas the nematodes were *Neoaplectana carpocapsae* Weiser (*Agriolos* strain) and *Heterorhabditis bacteriophora* Poinar.

Adult flies were inoculated with fungi by placing them for 1 minute on sporulating cultures of the fungi. They were then

Table 9. Results of attempts to infect *G. m. morsitans* with some insect pathogens.

Pathogen	Host stage	No. inoculated	No. infected
Beauveria bassiana	adult	20	6
	puparia	20	1
Hirsutella sp.	adult	20	0
	puparia	20	0
Metarrhizium anisopliae	adult	20	8
	puparia	20	1
Nomurea rileyi	adult	20	0
	puparia	20	0
Spicaria farinosa	adult	20	7
	puparia	20	0
Neoaplectana carpocapsae	adult	15	15
	puparia	20	1
Heterorhabditis bacteriophora	puparia	20	0
Bacillus t. thuringiensis	adult	20	0
Serratia marcescens	adult	30	11



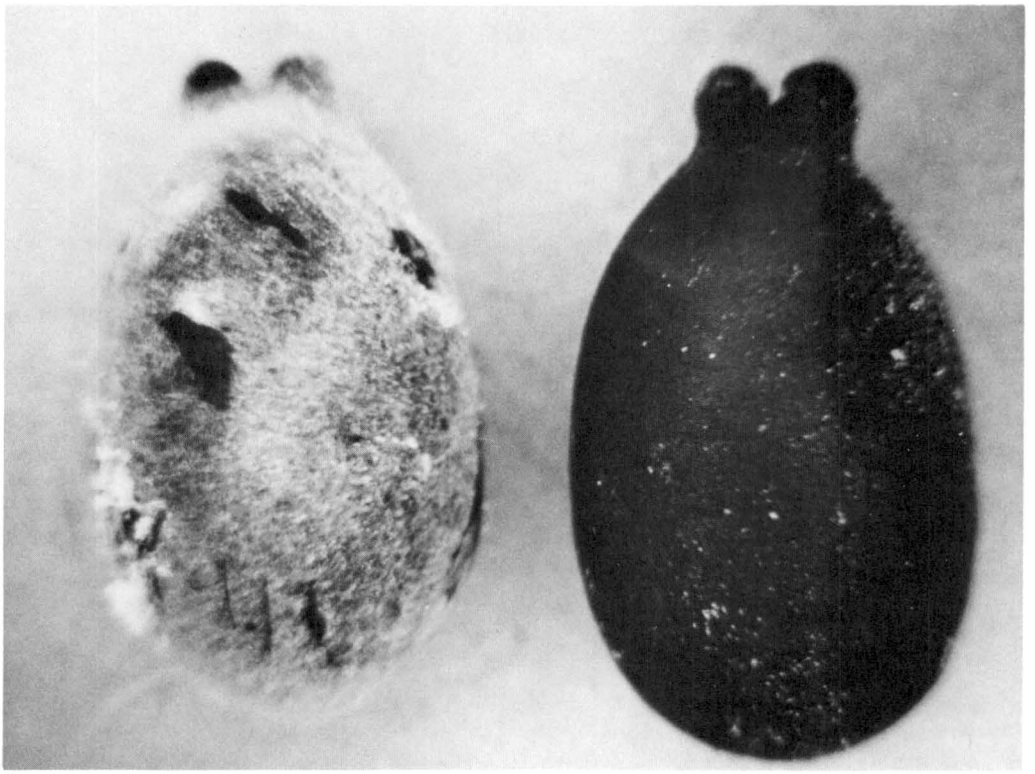
Adult G. m. morsitans infected with B. bassiana (G.O. Poinar, Jr).

held on petri dishes containing damp filter paper. Puparia were wiped over the surface of the fungal plates and placed in similar holding dishes. In trials with nematodes, puparia and adult flies were placed in petri dishes with damp filter paper and infective stage nematodes. Bacteria were first grown on plates of nutrient agar. Prior to inoculation, the plates were flooded with sterile water and a suspension was produced by mixing the bacterial cells and water with a brush. The suspension was then brushed on the ears of rabbits used for blood meals. Control flies were fed on the opposite uninoculated ear of the rabbit. After feeding for 4h, the flies were transferred to petri dishes containing damp filter paper. No attempts were made to infect the puparia with bacteria. All treated and control flies were held in environmentally controlled chambers at 30 °C and 70% relative humidity. Puparia were held until they showed visible signs of infection or the adult flies emerged. Adult flies were held until death, when they were then examined for the presence of pathogens.

Test Results

The results of the infection trials are summarized in Table 9. With fungi, about one third of the adult flies became infected with *B. bassiana, M. anisopliae,* and *S. farinosa*; the former two fungi each produced a single puparial infection. No infections were obtained with *Hirsutella* sp. or *N. rileyi.*

Surprising success was obtained with the nematode *N. carpocapsae* against the adult flies (Table 9). However, although the infective stages of both *N. carpocapsae* and *H. bacteriophora* were seen crawling over the puparia, only a single infection



Puparia of G. m. morsitans; one on left infected with B. bassiana (G.O. Poinar, Jr).

was obtained by the former species. Results obtained with *S. marcescens* were also surprising. Approximately one third of the adult flies that fed on rabbit ears previously treated with a bacterial suspension died 1–3 days after feeding. All dying flies had *S. marcescens* in their haemocoel. This was not the case for control flies, which generally died later than experimentally infected ones. No positive results were obtained with *B. thuringiensis.*

From these preliminary studies, it is obvious that the puparia of *G. m. morsitans* were almost completely resistant to the pathogens tested. This is not unexpected since, in nature, this stage remains in the soil for 3–4 weeks or longer. The only natural ports of entry are the respiratory openings in the polypneustic lobes. Apparently the infective stages of *N. carpocapsae* were able to enter these in one instance, but it would appear that the openings are very small.

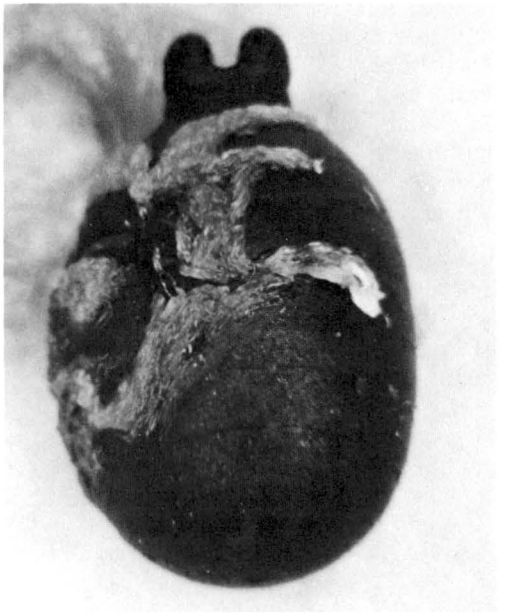
Under controlled conditions, however, adult flies proved susceptible to *B. bassiana, M. anisopliae, S. farinosa, N. carpocapsae,* and *S. marcescens.* That a certain proportion of them were infected with fungi is not too surprising considering that the species used were general pathogens that have been reported from a number of insects.

The bacterium, *S. marcescens,* which is composed of several strains, is widely regarded as a potentially useful pathogen. Although it is commonly recovered from diseased insects, it is generally thought to invade at the time of molt or at times of stress (when the insect is most susceptible). It may be that its successful penetration into adult flies indicates that the latter were under stress.

Adult infection with *N. carpocapsae* again demonstrates the ability of the nematode to enter non-oral openings of insects. Nematodes of this group have never been recovered from *Glossina* sp. in nature although mermithids have been reported from tsetse in both East and West Africa (IDRC 1974).

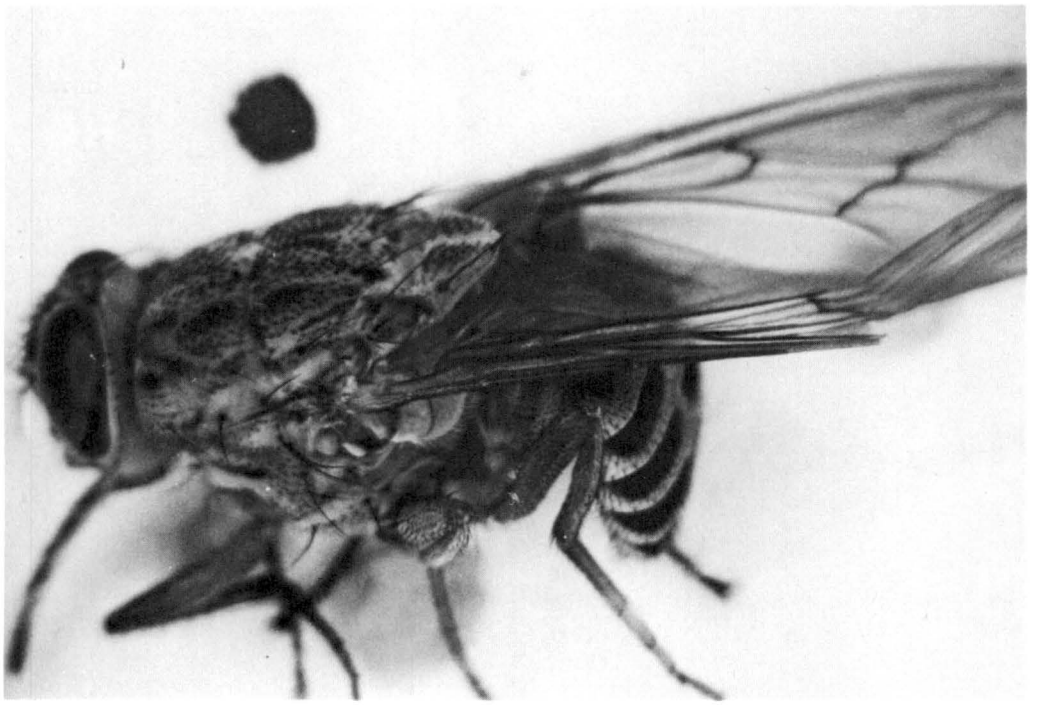
Our scanty knowledge of the natural pathogens of *Glossina* species is summed up earlier in this chapter and in IDRC (1974). Only the fungus, *A. repens*, and the bacterium, *B. mathisi*, have been reported as causing substantial mortality of puparia and adults, respectively, of *Glossina* species.

Application methodologies aside, the ability to grow selected pathogens *in vitro* will be a major consideration in the development of new microbial control procedures for use against tsetse. This ability is common to all of the pathogens that showed some degree of promise in this introductory study.

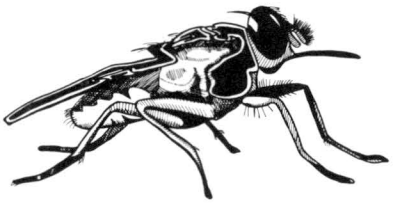


Puparium of G. m. morsitans opened to show the developing stages of N. carpocapsae (G.O. Poinar, Jr).

Adult G. m. morsitans containing S. marcescens (G.O. Poinar, Jr).



The successful use of a pathogen against any insect is dependent on many factors. Distribution of the pathogen is an important consideration because it must come in contact with the target organism. With pathogens such as the fungi and nematodes used here, the preferred sites of larviposition of the tsetse flies might be possible sites for inoculation. Thus the larvae, puparia, and emerging adults, which come in contact with the soil, clearly have opportunities to encounter a pathogen. Other possible uses of attractants or host contamination are rather speculative at this time. — G.O. Poinar, Jr., L. van der Geest, W. Helle, and H. Wassink.



Physiology

Larva and Puparium

The immature stages of the tsetse are remarkably well protected from potential pathogens, parasites, and predators. The larva spends its life inside the uterus of the mother fly, apart from a very brief period between larviposition and pupariation. The pupa is protected by the hard puparium that is itself normally protected by being buried in soil, sand, humus, or leaf litter. It would be unwise, however, to dismiss the larva and puparium from consideration, because nothing is known about the causes of larval mortality and little about pupal mortality.

The single egg hatches in the uterus of the adult fly and the larva is fed by the secretion of the milk gland. There are three larval instars. The 2nd and 3rd instars are characterized by the large respiratory (polypneustic) lobes at the posterior end. These lobes turn hard and black about 1.5 days before the larva is deposited. Larval development takes about 8 days at 30 °C; and 25 days at 18 °C (Mulligan 1970). When birth is about to take place, the larva undergoes peristaltic contractions, is probably assisted by contractions of the muscular uterine wall and abdominal wall, and leaves the uterus posterior end first.

It is assumed that the free-living larva neither feeds nor drinks, and certainly in many larval habitats there would be no opportunity to do either. However, the mouth is open and moist at birth and it may be a point of entry for microorganisms, particularly if the larva continues to make ingestive movements after deposition, even if only for a short period. Aborted larvae do continue to make ingestive movements and will take in liquid following birth.

The larva is deposited in a site that usually provides a medium in which the larva can burrow. If it is impossible for the larva to burrow it will eventually form a puparium on the surface of the ground. During the whole of its free-living existence the larva crawls or burrows incessantly (Finlayson 1967), with peristaltic waves passing along the body. In a suitable medium such as loose sand or soil, the larva begins to burrow at once. The orientation mechanism appears to be a photonegative response plus a strong thigmotactic response that direct the larva into dark crevices and soft substrates. The larva has a humidity response that takes it into the moister side of a chosen chamber in which the humidity of the air between sand grains is steeply graded. Although the larva prefers a moist atmosphere to a dry one, it is unable to burrow if the soil is too wet and has then to pupariate on the surface, where it may be exposed to a greater range of parasites and predators (Burtt 1952; Parker 1955).

The duration of the free-living larval stage ranges from about 15 minutes in ideal conditions for burrowing and finding darkness, to as long as 6 h when the larva is kept in the light with the minimum of tactile stimulation (Finlayson 1967). Maximum tactile stimulation in nature is provided by the medium into which the larva burrows. When a larva receives maximum tactile stimulation in darkness it pupariates in the shortest possible time. There is an integration of sensory input in which light intensity (or perhaps presence or absence of detectable light) and the amount of tactile stimulation are the two principal factors.

In the laboratory, a larva of *G. morsitans*

can crawl for at least 1.5 m, but no field work has been carried out to investigate the locomotory abilities of the tsetse larva under natural conditions. The larva certainly possesses the necessary locomotory and sensory abilities to move for short distances into darker, moister, and more tactile-stimulatory regions of the habitat in which it has been deposited. It is unlikely that the larva moves far in nature, but its efforts could provide the fine adjustments needed to complete the selection of the pupariation site, initiated by the mother fly. A journey of an inch or two could make the difference between death from over-heating caused by insolation warming of the soil or from desiccation in a habitat near the critical level for survival and development. Observations in nature (Bursell 1960a, Burtt 1952) indicate that the larva comes to rest between 0.6 and 7.6 cm below the substrate's surface.

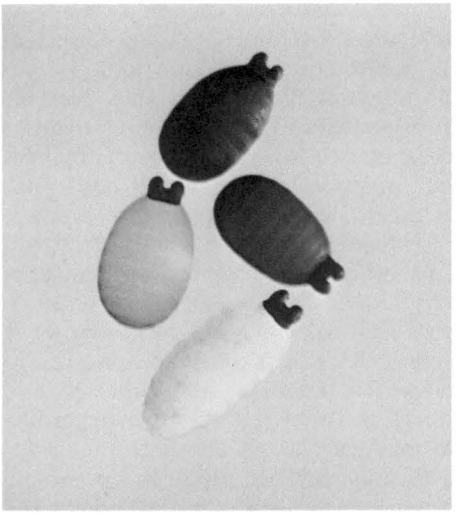
Pupariation follows a short period of circling and reversing (Finlayson 1967) that is terminated by the secretion of a clear liquid from the anus. This liquid was described by Kinghorn (1912). Evidence is presented by Lamborn (1915b) and Simpson (1918) that it is repellent to ants, which are probably among the chief predators of tsetse larvae and puparia. The liquid is slightly adhesive and causes sand grains to stick to the puparium and to each other. It may have the function of cementing a layer of sand grains together when the larva circles and reverses to form a chamber for the puparium, or at least to prevent collapse of the soil or sand until the puparium has hardened. Pupariation begins with head retraction, followed by barreling caused by muscular contractions. The cuticle undergoes molecular reorganization at this stage and is remodelled into the barrel-shaped puparium (Fraenkel and Rudall 1940). Hardening begins as soon as the barrel shape is assumed and proceeds rapidly through a range of tints from amber to pale brown within 10 minutes at 25 °C. Darkening continues for a day or so until the puparium becomes almost black.

Larviposition sites are chosen by the adult fly. Although there are variations in the types of site chosen by different species of tsetse, most have to satisfy two criteria: (1) the soil, sand, or humus must be dry and loose enough to allow burrowing; and (2) there must be shelter from the sun. In unshaded sites the temperature of the surface layer frequently reaches a level that would be lethal for a puparium. Bursell (1960c) and Pinhão (1970) reported that temperatures above 32 and 30 °C, respectively, are lethal to puparia of *G. morsitans*, the latter author noting that developing males are less tolerant of the higher part of the temperature range than are females. Larviposition sites are small areas (the breeding sites) within the larger area occupied by the particular species of *Glossina* (the breeding zone). In these breeding sites very large numbers of larvae may be deposited over long periods. In Rhodesia, for example, (Phelps et al. 1966) collected over 1 million puparia in 4 years from relatively small areas. In the Kariba region almost 60 000 were collected in September, 1965. It would be possible, therefore, for microorganisms to establish life cycles within the breeding sites.

From the above account of its life history, the tsetse larva would appear to be singularly well protected from pathogens, parasites, and predators. It feeds by sucking up the milk gland secretion that is poured into the uterus of the pregnant fly. To enter the larva via its food, microorganisms such as protozoa, bacteria, or viruses would have to pass through the body of the adult either into the uterus or the milk gland, or enter the reproductive opening and pass up the uterus and into the milk. There is no evidence at present that any of these routes of infection are exploited by pathogens. However, a possible line of investigation would be the study of aborted larvae and their mothers to see if abortion is ever caused by infection. It is usually assumed that abortions are caused by malnutrition (Glasgow 1963). Abortions at all stages in larval development occur frequently in laboratory cultures (Mulligan 1970), but little is known about their inci-

dence in nature except that they probably occur and that there may be an increase in incidence during the wet season (Jordan 1962b). The most likely time for infection of the larva to occur would be during the period between birth and pupariation. Obvious possible sites of entry of microorganisms are the mouth, the anus, and the polypneustic (respiratory) lobes,[10] but there are other small pores in the chemoreceptors of the antenno-maxillary processes and the sensory buttons (Finlayson 1972) that might give access to microorganisms.

It is not known whether mermithid larvae enter the larval, puparial, or adult stages of the tsetse. Foster (1963) reviewed the few records of mermithids that have been found in newly emerged laboratory-reared flies and suggested that they may have entered the adult flies from infested soil. However, the possibility that entry may have been made in the larval or puparial stage cannot be excluded. Laboratory and field work are required to solve this problem.



Larva, newly formed puparium, and two older puparia of G. morsitans showing progressive darkening (O.G. Harry).

Adult

At the moment of eclosion, the fully developed adult pushes off the anterior end of its puparium by the hydraulic ram-action of its eversible ptilinum. If it is buried under the ground, continued use of the ptilinum enables the insect to reach the soil surface. At this time the fly is ill-equipped to face the world, being pale, soft, and crumpled. However, it is able to run, and immediately seeks a suitable surface, usually vertical, upon which to begin the process of expansion. This it does by imbibing air and inflating the crop (a blind ending diverticulum of the foregut). The hydrostatic pressure thus exerted in the haemolymph leads to unfolding of the cuticle, assumption of the adult shape, and expansion of the wings. Hardening and darkening of the cuticle then follow. Although these processes are not completed for several hours, the adult insect is able to fly approximately 1 h after emergence.

Depending upon the extent of its nutritional reserves (mainly lipids — see Bursell 1960c), the teneral adult may initiate host-seeking behaviour between 1 and 2 days after emergence. Teneral flies deliberately deprived of food do not generally live beyond the fifth day (Brady 1973). Before feeding, the thoracic flight musculature of the adult is poorly developed, but in wild flies it develops rapidly over the first two hunger cycles (Bursell 1961). Development is slower in flies held in captivity (Bursell 1961; Langley 1970), and there is evidence that flight exercise itself stimulates such muscle development (Bursell and Kuwengwa 1972).

During the early days of adult life, *Glossina* spp. are vulnerable to attack from predators. The ingress of certain parasites or pathogens may be possible via the cuticle or the spiracular openings of the respiratory system before hardening and darkening are completed, although no evidence

[10] See footnote p. 87.

of this has yet been obtained (but see p. 90, 91).

A combination of adenotrophic viviparity, and a fast-flying adult stage has enabled tsetse flies to achieve a high degree of independence from their hosts, in spite of their obligatory haematophagous habit and their complete dependence on vertebrate blood as a source of nutrients. Unlike many other haematophagous arthropods that will feed from a wound, or other free blood surface, the adult tsetse must first pierce its host's tissues with its needle-like mouthparts before it can imbibe. This necessarily restricts the range of organisms that may gain access to the alimentary canal during feeding. There is no evidence that adult tsetse ever suffer ill-effects from microorganisms originating on the host skin surface.[11]

Tsetse will readily imbibe a variety of liquids presented under stretched parafilm membranes (which the flies probe in response to a temperature stimulus) provided that the liquid contains adenosine-5'-triphosphate (ATP) in solution (Galun and Margalit 1969). High energy phosphates are phagostimulatory for a number of blood-sucking insects, but their exact role in stimulating ingestion of blood is not yet clear (see Langley 1976). In *Glossina*, the type and texture of the membrane are important in stimulating blood ingestion in an *in vitro* feeding system (Langley and Maly 1969), a dual membrane of agar and stretched parafilm being most successful (Langley and Pimley 1976; Mews et al. 1976). Recent studies with such membranes have shown that *Glossina* spp. have little ability to discriminate between solutions of varying osmotic pressure and ionic composition, some of which are rapidly lethal to the insect (Langley and Pimley 1973). Toxic solutions are also readily ingested through these membranes even in the absence of ATP (Langley, unpublished data).

It is now well established that a variety of bacteria will multiply within the alimentary canal and haemocoel, following ingestion of infected blood through membranes, and that these flies die (Langley 1972; Nobre and Santos 1970; Oliveira and Nobre 1970). It has also been demonstrated that these bacteria can be serially transmitted to other flies following feeding through the same membrane upon the same blood pool and that this transmission is most likely achieved via the salivary gland secretions of the infected flies (Bauer 1974). These investigations, coupled with the observation that a very low concentration of sulphonamides in the diets of laboratory host animals will effectively reduce the reproductive rate of tsetses (Jordan and Trewern 1973), serve to illustrate the sensitivity of the fly to the composition or contamination of its food.

The feeding habits of tsetse flies in their natural environment make it unlikely that detrimental microorganisms would be ingested with their blood meals. Nevertheless, the apparent lack of "taste" discrimination, and the ease with which the flies can be adversely affected by ingestion of bacteria or toxins opens up an avenue of investigation that may have prospects in the development of biological control techniques.

The tsetse larva is entirely nourished *in utero* by milk gland secretions of the pregnant female fly. Thus, survival of the immature stages is dependent upon survival of the adult female. Smaller than normal offspring are produced if the female is undernourished, or her pattern of feeding behaviour is interrupted.

A giant-cell zone exists at the junction of the anterior (water absorbing) and middle segment (digestive portion) of the midgut in adult *Glossina*. These cells contain "bacteroids" that are thought to provide a synthetic function similar to the microorganisms present in the alimentary canals of a number of other haematophagous arthropods. An exact function has yet to be ascribed to them, although they do appear to be essential for survival. If the bacter-

[11] But see information of Roubaud and Treillard 1935, p. 78 (ed).

oids are destroyed by antibiotics the fly dies (Nogge 1974). It is essential that these bacteroids be transmitted to succeeding generations of tsetse since they have no known external origin. At present two hypotheses exist: (1) the bacteroids are transovarially transmitted (Huebner and Davey 1974); and (2) they are transmitted via the milk gland and ingested by the feeding larva (Ma and Denlinger 1974). Whichever hypothesis proves to be correct, the evidence is that a microorganism has the ability to pass from an intracellular position in the alimentary canal, to cross the haemocoel of the adult fly, and to enter the body cavity of the developing offspring. Thus there exists yet another pathway for the possible exploitation of biological control, using suitable organisms.

Pregnant female tsetse flies feed more frequently and ingest larger meals than males, except toward the end of the period of larval growth, when the third instar larva occupies most of the available space within the distended female abdomen. Immediately after feeding (a rapid process, during which two or three times its own body weight of blood may be ingested in one minute), the adult fly seeks a resting place. During the next hour, it eliminates a large volume of water from the blood meal via the excretory system. Diuresis is a rapid process (Bursell 1960b) but composition of the urine produced is regulated by the Malpighian tubules and the rectal reabsorption mechanism (Gee 1975). Freitas et al. (1969) reported that newly emerged *G. morsitans* retain haemoglobin longer than older flies. Rapid diuresis has survival value in that a fully fed insect is slow flying and therefore vulnerable to predation. A female fly containing a fully grown third instar larva, whose live weight at birth exceeds that of its mother, is also ponderous. These females do not feed, and are rarely attracted to catching parties in the field. They, too, must be more vulnerable to predation.

After feeding, and elimination of excess water through the wall of the anterior segment of the midgut, the blood meal enters the middle or digestive portion of the midgut where it is subjected to digestion by the enzyme trypsin (Gooding 1974). The final products of digestion are absorbed across the wall of the posterior segment of the midgut, whereas indigestible haematin is passed on to the hindgut. Here it meets with the major excretory product, uric acid, from the Malpighian tubules. Water and salts are reabsorbed in the rectum, and waste is voided to the exterior as a semisolid paste (Bursell 1965).

The rate of transfer of an ingested blood meal from the crop (which is simply a temporary storage organ) to the midgut is thought to depend upon the rate of growth of the peritrophic membrane, which is a closed sleeve originating from special secretory cells around the proventricular valve (Wigglesworth 1929). The peritrophic membrane begins to grow in a young adult fly before it obtains its first meal. Depending upon the extent of its growth at the time of feeding, the blood meal will spend a longer or shorter time in the crop. Harmsen (1973) has associated the length of time that a blood meal spends in the inert environment of the crop of *G. pallidipes* with isoenzyme transformations in *Trypanosoma brucei* ingested with it. If the blood meal is transferred too soon, as in an older fly whose peritrophic membrane has already grown considerably, the trypanosome is faced with the hostile environment of the midgut before it has adjusted biochemically. This argument is used to explain the infectibility of very young adult *Glossina* with *T. brucei* contained in the first blood meal, and the decreased infectibility of flies that are some hours older when obtaining their first blood meal, or subsequent meals (Harmsen 1973).

A different explanation is offered by Laveissière (1975) for the infectibility with *T. brucei* of *Glossina* adults ingesting their first meal as opposed to subsequent meals: he observed that before ingestion of the first blood meal the peritrophic membrane grows and curves back on itself within the

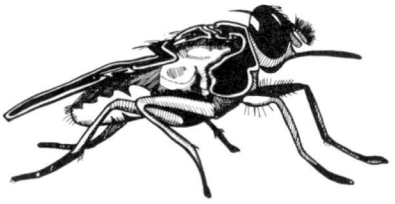
midgut. The fold restricts its volume and the peritrophic membrane ruptures with ingestion of the first meal, allowing trypanosomes to enter the ectoperitrophic space where they continue development. With subsequent meals the peritrophic membrane is not folded and forms a continuous sleeve passing into the hindgut, thus preventing access to the ectoperitrophic space. Again, whichever hypothesis is correct, the fact remains that physiological differences exist between older and younger unfed adults such that younger flies are more susceptible to *T. brucei* infections. Such susceptibility of the young adult fly may have relevance in future strategies for biological control using microorganisms.

The anus of the adult fly is clearly a possible site of entry for potential pathogens, although none has been recorded. Frequent flushing of the rectum with large volumes of fluid immediately after feeding, and production of the semisolid faecal pellets between meals render the possibility of infection by this route rather remote.

A single mating resulting in the transfer of spermatozoa in a gelatinous spermatophore to the female oviduct (Pollock 1970) generally suffices for the whole of the female reproductive life. Spermatozoa leave the spermatophore (which is subsequently ejected by the female) and make their way via the paired spermathecal ducts to a pair of hollow chitinous spheres known as spermathecae from where they fertilize successive ova, ovulated singly, each 9 days or so at 25 °C following successive larvipositions. To some extent the female genital opening communicates with the external environment, since, as far as is known, the developing larva *in utero* respires aerobically via its paired posterior respiratory lobes. This is therefore another potential site of entry for pathogens.

Copulation lasts from several minutes to some hours, and there is evidence that a certain minimum time spent in copulation is necessary for correct spermatophore delivery and for the physiological events leading to ovulation of the first mature egg by the female fly to be entrained or initiated (Saunders and Dodd 1972). The flight capability of copulating pairs of *Glossina* is clearly impaired, and vulnerability to predation is probably increased at this time. The male fly manufactures his spermatophore from accessory gland secretions (Pollock 1974) that are generally not fully developed until after digestion of one or more blood meals. In considering possible techniques of biological control, therefore, the transfer of material between the sexes should not be overlooked, although, again there is no evidence that pathogen transmission is achieved in this way.

It seems that the only life stages vulnerable to large-scale predation or parasitization are those spent in the soil within the puparium. The larva spends only a very short time outside the body of the adult female, during which time it seeks a suitable pupariation site and does not feed. The adults are active fliers, existing at relatively low densities over wide areas. Their feeding behaviour and monotonous diet provide them with considerable protection from chance infections via the alimentary canal, which may account for their surprising sensitivity to variations in diet composition and to infection with a variety of bacteria under experimental conditions in the laboratory. It is too much to hope that this susceptibility is the "Achilles' heel" of the tsetse but there is clearly scope for intensified research on novel approaches to biological control, based upon a sound knowledge of the physiology of the genus, as well as a closer study of the physiological relationships between tsetse flies and their known predators, parasites, and pathogens. — L.H. Finlayson and P.A. Langley.



Distribution and Bionomics

The present distribution of the known species and subspecies of glossinids is strictly African. They are absent from the Malagasay subregion and their presence in a small enclave of southwest Arabia is questioned. Covering an area of 10.8 million km^2, they formerly occurred farther to the north and to the south, but their distribution became more restricted under the influence of ancient climatic changes. Their northern limit is close to 14 °N. South of the equator, the boundary is a line joining Benguela, Angola, to Durban, South Africa (Ford 1963).

On the basis of climate, four main vegetation zones may be distinguished in Africa: (1) the dense rain forest; (2) the savannah-forest mosaic; (3) the Guinean Savannah woodlands; and (4) the Sudanian Savannah woodlands. Tsetse flies are distributed throughout these large bioclimatic zones, but are absent from the Sahelian zone and from the deserts of the north and the south (Sahara, Kalahari). The major factors governing their distribution are: climate (temperature, rainfall); vegetation; fauna; and human activities. Potts (1953–54) mapped the general distribution of *Glossina* spp. Some, living in a very wide range of environments, have a vast distribution. Others, living under a narrower range of conditions, have a more restricted range.

Fusca Group

With the exception of two species (*G. brevipalpis* and *G. longipennis*), these flies are associated with the zones of the equatorial forests. Most species occur in central and west Africa. Ford (1971) distinguished three subgroups according to their distribution:

(1) Species from the evergreen forest: *G. tabaniformis, G. haningtoni,* and *G. nashi.* They occur in the Congo basin, in the forests of Gabon and of south Cameroon, and on the coast of the Gulf of Guinea to Liberia (*G. nigrofusca*).

(2) Species from the relict forest — between the forest zone and the savannah woodland: *G. fusca* in western and central Africa (western part of Uganda); *G. medicorum* in western Africa; *G. fuscipleuris* in eastern and central Africa; *G. schwetzi* in central Africa, *G. severini* in the eastern part of eastern Africa; and *G. vanhoofi* in central Africa.

(3) Species of secondary forests of east Africa: *G. brevipalpis* gives an indication of the past extension of the evergreen forest in eastern Africa.

G. longipennis lives under much more arid conditions than the other species (subdesert conditions) north of Tanzania, Kenya, the Somali Republic, and Ethiopia. It is the most xerophilic tsetse.

Morsitans Group

Tsetse flies of this group are associated with the savannahs, surrounding the evergreen forests of central Africa, that replaced the ancient forest of eastern Africa. These savannahs extend from the forest to the desert or to the sea.

G. m. morsitans occupies the *Brachystegia* savannah in eastern Africa (Mozambique, Rhodesia); *G. m. orientalis* occurs in the same type of vegetation, but

Tsetse bush (Acacia spirocarpa dominant — G. swynnertoni prevalent) near Magugu, Tanzania, 17 March 1962 (M. Laird).

in Zambia (west of Tanzania–Shaba) and Angola; *G. m. submorsitans* extends from north of Uganda, to southern Sudan to Guinea and lives in the *Isoberlinia doka* savannahs. *G. pallidipes* and *G. longipalpis* are two closely related tsetse species, *G. pallidipes* occurs in eastern Africa, from the forest edges to the more arid thicket zones, *G. longipalpis* replaces *G. pallidipes* west of 17 °E occupying more humid areas in western Africa. *G. swynnertoni* is restricted to the north of Tanzania and to the southwest of Kenya where it lives in a wooded *Acacia* steppe; and *G. austeni* is limited to a narrow, discontinuous zone of the coastal plains of eastern Africa, from the southern part of the Somali Republic to South Africa. It lives in secondary thickets and bushes at altitudes above 200 m.

Palpalis Group

The species of this group occur in the hydrographic system associated with the Atlantic Ocean and the Mediterranean. Machado (1954) studied the distribution of the species and subspecies.

The general area of *G. palpalis* (*sensu lato*) is a narrow coastal band in western Africa, exceeding 5000 km in length and extending from Senegal to Angola (13 °S). The widest part of this band is in Nigeria where it reaches 800 km extending from the Sudan savannahs to the forest.

G. p. gambiensis occupies the northern part of the area (from Senegal to the Ivory Coast); *G. p. palpalis* occurs in the central and southern parts, the two subspecies being separated in the area of Togo/Benin. *G. palpalis* (*s.l.*), a rain-forest fly, adapts itself quite easily to drier conditions; *G. fuscipes* is also a forest species, but it shows greater preference for dense waterside forests than *G. palpalis*. Its distribution area is immense in central Africa — the entire Congo basin, reaching the Nile basin and the shores of Lake Victoria; *G. f. fuscipes* occupies the central and northern parts of the area of distribution of the species; *G. f. quanzensis* is a tsetse of the Kasai basin, *G. f. martinii* occupies the Lake Tanganyika basin; *G. tachinoides* is distributed almost entirely in western and central Africa

with a discontinuity at the level of Sudan from which it is absent. It occurs only west of Ethiopia and lives among *Mimosa* sp. and *Salix* communities and the forest galleries bordering streams; *G. p. pallicera* and *G. p. newsteadi* belong to the great coastal forest of the Gulf of Guinea and of the Congo basin; and *G. caliginea* has a very limited habitat (littoral forest of Nigeria, Cameroon, Gabon) because a very high relative humidity is essential to its survival.

Activity

Tsetse flies are diurnally active. The factors determining their mobility are: (1) their search for food — a blood meal taken from a vertebrate; (2) the male's search for a mating partner, followed by the female's search for a larviposition site; and (3) the search for resting places. The physiological aspect of activity has been reported by Bursell (1961) who recognized in *G. swynnertoni* four phases of activity in relation to stages of digestion and reconstitution of fat reserves. Not only physiological factors but also external ones modify this behaviour: temperature, relative humidity, and luminosity.

Harley (1965a) expressed the activity cycle of tsetse as relative numbers caught by hand or in traps at different times of day. Tsetse flies coming to traps or catchers represent only part of their active population. This leads to the concept of availability.

Hourly records of capture reflect diurnal variations in the activity of each species. From numerous observations made on different species of the subgenus *Nemorhina*, Challier (1973) derived the following general pattern: (1) appearance of tsetse at sunrise; (2) progressive increase of attacks to a morning maximum; (3) slackening of biting activity in the middle of the day; (4) vespertine maximum of activity; (5) rather sharp decline in the evening; and (6) cessation of activity at dusk.

In the male *G. p. gambiensis*, two phases of activity were observed between sunrise and sunset: one in the morning, the other in the evening, with a maximum between 12:00 and 13:00. The absolute maximum occurred between 10:00 and 11:00 in the savannah zone, and at the beginning of the afternoon in the forest zone.

Except in May and June, the aggressivity curve is not as clear for the females. They are active only while searching for a host. At other times, they are resting. By contrast, the males come to the catchers not only when they are hungry, but also when they still have in their alimentary tracts nondigested blood. More rarely, they come when in an engorged state. Their activity appears therefore to have a greater importance.

At the beginning of the cycle, *G. p. gambiensis* is active when two conditions are realized simultaneously: (1) temperature above 16 °C; and (2) light intensity close to that observed at sunrise.

At the end of the cycle, light appears to be the limiting factor. When searching for a determining climatic factor, one realizes that the aggressivity curve of *G. p. gambiensis* neither follows that of temperature nor of the saturation deficit. The activity maximum does not correspond to the maximum of these factors, but appears to be related to light.

Harley (1965a) studied *G. f. fuscipes* and found that the activity cycle of the two sexes showed a peak during the midday hours varying, according to region and season, from 10:00 to 16:00.

In *G. tachinoides* the daily activity curve varies also with the season. However, activity maxima are observed at temperatures and light intensities that, regardless of season, are rather uniform. Relative humidity, while not affecting the intensity of activity, seems to have an influence on its duration (Gruvel 1974a). Diurnal variations are represented by three types of curve, depending on the time of year: (1) distinct maximum at midday: November, December, January, and February; (2) plateau with maximum only slightly apparent: early May, August, September, and early October; and (3) two maxima, morn-

ing and late afternoon: February, March, April, June, and early July. Nash (1937) established the limits of activity of this species in Nigeria between 15.5 and 41 °C, the maximum being between 24 and 31 °C.

G. p. palpalis on the fringe of the forest zone shows a constant activity from 11:00 to 16:00 during the rainy season and two maxima between noon and 17:00 during the dry season, with a slight slowing down about 15:00 (Page 1959).

G. fusca shows a peak of activity just before dusk during the rainy season (Jordan 1962a). *G. m. orientalis* attacks mainly in the morning between 6:00 and 8:00, but to some extent in the afternoon. By contrast, *G. longipalpis* is active 2 h after sunrise. This morning peak is followed by a rapid decline, and by renewed activity between 16:00 and dusk. Nash (1937) was able to demonstrate a cycle closely related to temperature for *G. m. submorsitans*.

Temperature appears to play a dominant role in determining the type of diurnal activity, but the onset of tsetse activity depends on both adequate temperature and light conditions.

Although the active phase of the tsetse cycle is diurnal, species have been observed both flying and attacking at night: *G. longipennis* (Lewis 1950), *G. pallidipes* (Moggridge 1948), and *G. brevipalpis* (Harley 1965a).

Dispersal

Numerous marking and release-and-recapture experiments have been undertaken to shed light on this phenomenon. Such studies are important for the understanding of population dynamics and epidemiology. Jackson (1941) defined, under the term "ambit," the rather well defined area within which a tsetse adult moves during its lifetime. When tsetse fly about, there is a linear dispersal in the case of riparian populations and a surface dispersal in the case of savannah ones. Glasgow (1963) also studied this problem. Challier drew attention to the occurrence of both immediate dispersal within the ambit, and distant dispersal beyond it.

Linear Dispersal

From a release point in a forest gallery, it is enough to study sections of this gallery longitudinally to determine the movements of marked tsetse after release. This also gives a measure of the speed and amplitude of the flies' movements. In general, *Glossina* disperse outside the forest gallery. In this type of dispersal, authors agree on the existence of preferred flight lines along open spaces (e.g. paths and game tracks, called "ecological corridors" by Gruvel).

G. p. gambiensis moves in its ambit via these open flight paths in search of a host. It flies along the shores of streams, and along edges of forests. Gibbins (1941) mentioned a movement of 8 km in 24 h. An appreciable proportion of the flies may leave their ambit and cross sparsely wooded areas, thus embarking upon a distant dispersal. The flies may leave a site in which conditions have become adverse and search for one more favourable. In northern Nigeria the longest observed displacement of *G. p. palpalis* was 5 km, females dispersing as widely as males. Dispersal in galleries of *G. tachinoides* is accomplished at the rate of 1 km/day.

Gruvel demonstrated seasonal migrations of *G. tachinoides* in Chad. Some sites were occupied in a cyclical fashion in relation to hydrography and climate. Dispersal was more extensive during the rainy than dry season, the latter being the time at which the area of distribution of the flies diminished. Nash and Page (1953) recorded movements of 5 km for this species in northern Nigeria. Van Vegten quoted a 5 km displacement of *G. f. fuscipes* along rivers.

Surface Dispersal

This is the type of dispersal of savannah or large-forest flies. During the dry season,

the *G. morsitans* population leaves the savannah and concentrates closer to the rivers. Later on, during the rainy season, they disperse again, enlarging their area of distribution. This movement may attain 5 km/day in the Sudanian Savannah zone, and 15 km in the Guinean Savannah zone (Davies 1967).

For *G. morsitans* and *G. swynnertoni*, Jackson (1937) distinguished between real habitats, where glossinids may be found resting, and open feeding grounds, where flight and visibility are facilitated. However, Pilson and Pilson (1967) discussed this concentration phenomenon in *G. morsitans* during the dry season by comparing two sampling methods — capture with a net versus capture with animal bait. They showed that the female population in particular is well dispersed among the different types of vegetation. This contradicts the "feeding-ground concept." Besides these different types of movements, the ease with which tsetse are passively transported by an animal, a road vehicle, or a canoe must be noted. Dame et al. (1975) have recently demonstrated that Langford laboratory-bred males of *G. m. morsitans*, despite 2 years of colonization and lengthy air-transportation, have "survival and dispersal characteristics similar to those of the native males in the natural habitat."

Resting Sites

Rest is a very important period in the life of *Glossina* because the daily activity period is estimated at only half an hour (Bursell 1959). The search for a resting place is therefore a positive act aimed at finding a site where the microclimate is in conformity with the requirements of the species (Challier and Laveissière 1971). Tsetse choose a resting place in order to: (1) protect themselves against extreme climatic conditions; (2) digest a meal of blood; and (3) lie in wait for a host.

Davies has distinguished real diurnal or nocturnal resting places from watching places. In their true resting places, the flies remain totally immobile or indifferent. They are then in a state of complete rest. In watching places, they poise at the ready during their activity period, waiting for a suitable host. Their homochromy renders them difficult to detect, and marking techniques have proved essential to the discovery of their nocturnal resting places (e.g. fluorescent powders, and reflecting paper glued to the thorax).[12]

Diurnal Resting Places

The flies rest at various heights on different types of vegetation (tree trunks, branches, twigs, either surface of leaves). Different factors (temperature, humidity, luminosity, wind) may cause tsetse to change their resting place during the day.

G. tachinoides rests during the day on the lower parts of trunks and of low branches, and in tree-trunk cavities. Resting sites are always located in the shade and are protected against the wind. Their height from the ground (0–4 m) decreases with an increase in the environmental temperature. No flies are found at a resting place where the temperature is over 36 °C. Humidity at the level of the site is always above that of the surrounding air (Gruvel 1974a). While settled, *G. tachinoides* always remains with its head directed upwards. Jewell (1956) noticed no differences between males and females in the choice and the height of resting places. MacDonald (1960) found that 50% of *G. palpalis* rested between the ground and a height of 30 cm, leaves and twigs being their preferred sites; whereas, most *G. f. fuscipes* were found between 0–1.5 m on twigs, leaves, and low branches by Glasgow (1967).

G. m. submorsitans rests at the base of trees where the temperature is the lowest (Nash 1937). The lower surface of horizontal branches is preferred at temperatures

[12] New instrumentation, such as the Night Vision Device based on the light-accumulation principle, may have something to contribute here (ed).

below 29.5 °C, and 68% of the population rests at a height of between 0 and 30 cm. Engorged flies keep their heads upwardly directed immediately after the blood meal is taken, usually resting at a height of about 1 m. Later they climb to 2–4 m and rest with their heads directed downwards (Nash 1952). Langridge et al. (1963) reviewed the resting places of nine glossinid species: *G. swynnertoni, G. morsitans, G. longipennis, G. brevipalpis, G. pallidipes, G. austeni, G. fuscipleuris, G. palpalis,* and *G. tachinoides. G. swynnertoni* rests on the lower surface of small branches from 1.8 to 5.6 m above the ground (Jackson 1946).

Nocturnal Resting Places

Nocturnal detection of resting flies is made easier by their having been marked with fluorescent powders, paints, or reflecting paper.

The resting places of *G. tachinoides* are occupied as soon as darkness sets in and are provided by the upper and external parts of the arboreal foliage (Gruvel 1974a). *G. p. gambiensis* chooses nocturnal resting places near the ground and close to water. The flies settle in the lowermost parts of the vegetation, perching on the extremities of low-growing plants. The population is concentrated in a central nucleus, 10–20 cm in height and 50–75 cm in width, starting at about 50 cm from the edge of a stream. Tsetse are undoubtedly attracted by the CO_2 concentrated in the lower layer of the atmosphere, independent of temperature and humidity, which show little variability. The nocturnal resting places of *G. morsitans* are provided by the tips of leaves and twigs (MacLennan 1961), the ground, grass, and tree trunks (Robinson 1965). At sunset *G. swynnertoni* moves from the lower surface of branches to leaves, according to Isherwood.

Food Preferences

Both sexes of *Glossina* spp. are haematophagous. Analyses of the blood found in the digestive tract of captured flies permit (within limits) the identification of their preferred hosts. Earlier methods of identification of blood meals were based on microscopic examination of the stomach contents of the flies. This allowed only a very broad classification, e.g. on the basis of bird and reptile erythrocytes being oval and nucleated, those of Camelidae oval but without nuclei. The red blood cell diameters also enable the identification of the blood of some mammals (Lloyd et al. 1924). This method requires relatively fresh blood meals, and is further complicated by the fact that tsetse bite again at various stages of their feeding cycle, making it difficult to reach satisfactory conclusions because many of the blood cells are no longer fit for a precise determination.

Investigation of these meals by means of immunological tests gives much more accurate information on the feeding habits of *Glossina*. The digestive tracts of the dissected flies are squashed onto filter paper and kept dry for study (Weitz and Glasgow 1956). Identification by the precipitin test is first carried out at the vertebrate-group level; the haemagglutination inhibition test then enables one to determine the species within the group (Weitz 1952).

After analyzing more than 22 000 blood meals from 15 tsetse species, Weitz (1963) obtained important information on their food preferences. In this manner, he determined five main host groups.

Suidae

In Kenya and Tanzania, 65% of the blood meals eaten by *G. swynnertoni* come from the warthog. The preferred host of *G. austeni* is the bush pig; *G. fuscipleuris* feeds mainly on the giant forest hog. In western Africa, *G. tabaniformis* feeds on the bush pig 70% of the time. However, large ruminants may also be chosen: giraffe 8%, buffalo 8%, and hippopotamus 2%.

Suidae and Bovidae

In this group 30–45% of the meals had

their origin in Suidae, and 25–30% in Bovidae. *G. m. morsitans* feeds mainly on the warthog, zebu, and buffalo. The other two subspecies have similar feeding habits, but with an important proportion of blood meals taken on the greater kudu for *G. m. orientalis*, and on the hartebeest for *G. m. submorsitans*. Some animals (zebra, impala), which are present in the distribution zone of these flies, are neglected.

Bovidae

In eastern Africa *G. pallidipes* and *G. longipalpis* have similar habits, the bushbuck representing 80% of their choices. The bushbuck accounts for 74% of the meals eaten by *G. fusca* with the balance being taken on the bush pig and the aardvark.

Mammals Other than Suidae and Bovidae

G. longipennis feeds mainly on the rhinoceros (60%) but also on the elephant (12%). It is the only tsetse for which the ostrich is of some importance as a host (7%) (Weitz et al. 1958). *G. brevipalpis* feeds mainly on the hippopotamus.

Miscellaneous Hosts

These include man and the other common inhabitants of the region under consideration: primates, bovines, crocodiles, and monitor lizards. Tsetse of the subgenus *Nemorhina* belong to this group. Baldry (1964a) noted that in the immediate vicinity of dwellings, *G. tachinoides* prefers to bite pigs.

The various species of *Glossina*, therefore, each have a particular mode of feeding. They show varying degrees of host-preference, and a capacity for selecting a replacement host in the absence of the preferred one.

Sampling

The objectives of sampling are to measure natural population sizes and to study their composition: sex ratios, ages of the individuals caught, longevity, etc. Morris (1955) described two types of estimates: (1) absolute estimates, consisting of counting the number of animals per unit of sampling, as an example in a known fraction of the habitat; and (2) relative estimates, comparing captures in space and time, and measuring population in units of unknown size. The methods used with tsetse flies are of the second type, although attempts at absolute estimates have been made by marking, release, and recapture.

A natural tsetse population is made up of adults and puparia (the free larval stage is very short). A sampling method should account as closely as possible for the total individual composition. One method alone will not suffice, since it will apply to only one part of the population. Summarizing observations by several authors, Gruvel (1974a) concluded that generally: (1) bait animals (young bovines) allow the capture of more flies than any other method; (2) starved flies are more readily attracted by traps or bait animals; (3) a larger number of females are caught with traps (those that are, are the older or gravid ones); and (4) the adults caught by hand are mainly young ones and males in search of the opposite sex — starved flies and gravid females are much less common. No single sampling method is therefore entirely adequate and the results have to be interpreted very carefully.

Preimaginal Populations

Studies have been mainly qualitative, describing puparial sites of the different species of *Glossina*. Concentrations of puparia have sometimes been recorded. In general, though, puparia are widely scattered. Although usually buried in sandy soil, they sometimes occur on the surface under leaves. Generally, they occur in areas protected by a permanent shadow (fallen trees, cavities in the soil, or trees).

Jackson (1937) estimated at 12 000 the total population of *G. morsitans* in 885 ha. No adequate solution has yet been found for the sampling of puparial populations,

Searching for G. tachinoides puparia, Chad, April 1967 (J. Ford).

Pathfinders picking up tsetse fly specimens, Botswana (WHO photo, J. Abcede).

in spite of its great interest for the study of population dynamics.

Imaginal Populations

Glasgow (1963) defined four sampling methods in order to find out if numbers of tsetse vary with time, if they are peculiar to certain zones, and what is the composition of the population.

Rounds

Using nets with short handles, the samplers traditionally catch the flies alighting on them while following fixed itineraries (the "fly-rounds") of known lengths. Such an itinerary may have been chosen in such a way that it crosses in turn all the vegetation types of one area. They may be as long as 10 km. More recently these were replaced by linear transects with halts every 25–100 m (transect fly-round; Ford et al. 1959). Capture methods by rounds were mainly used for *G. morsitans* and *G. swynnertoni* whose habitat is spread out in savannahs. In his catching circuit, Jackson (1946) used a rectangular spiral, Glasgow, an octogonal spiral. Van den Berghe and Lambrecht (1962) sampled along the 100 m sides and the diagonals of experimental squares.

Fixed Captures

These methods are especially useful in demonstrating the daily variations in fly activities. A bait animal is sometimes used at the location of capture (fettered animal). This method has been used for *G. pallidipes* (Smith and Rennison 1961a, b, c; Harley 1965a), *G. brevipalpis* (Harley 1965a), and *G. fusca* and *G. tabaniformis* (Jordan 1962a). Morris (1961b) studied *G. p. gambiensis* in northern Ghana and noted that fly-round parties in low densities capture only one-fifth of the numbers caught by stationary parties. In general, the fixed station method is preferable for the study of forest gallery tsetse (*G. palpalis, G. fuscipes*) to that of rounds applied to *G. morsitans*.

Capture of Resting Flies

This method is necessary for obtaining data on the post-engorgement period. It is also the only way to determine the precise location of diurnal or nocturnal tsetse resting sites. Pilson and Pilson (1967) allowed individuals of *G. morsitans* to gorge themselves prior to marking and releasing. Nash (1952) was able to locate the resting places of *G. medicorum*.

Traps

A large number of types of traps have been described and used for catching tsetse flies. Members of the *palpalis* group, and *G. pallidipes*, are the ones that come the most readily to traps. In the case of *G. pallidipes*, caught mainly with the Langridge (1968) trap, more females than males are caught. The location of the traps is very important — Glasgow estimated that a trap was no longer attractive beyond 100 m. Bait or chemical attractants increase the yield of the sampling method (see following section). — J.P. Eouzan.

Trapping Technology

The utilization of traps for catching animals predates recorded history. Southwood (1968) has distinguished between traps that catch animals at random, and those that catch them after having attracted them with a device of some kind.

Tsetse traps belong to the second category. Depending on the principle of their functioning, they may be classified into: (1) attracting screens or panels with or without adhesive; (2) falling cages and artificial refuges; and (3) traps proper ("tridimensional" in Buxton 1955).

The history of tsetse trapping began when Maldonado (1910), a planter from Principe Island, had his workers wear on their backs a black piece of cloth covered with glue. The first real tsetse trap was constructed in Zululand by Harris (1930). Later research workers have used this model as a basis for numerous variations.

Screens

Screens are attracting surfaces that may be either mobile or immobile. Following Maldonado, Lamborn (1915a) and Ruttledge (1928) tried various adhesive substances, but with little success. Swynnerton (1936) placed an electrified screen on a vehicle.

The presence of a screen in a crew of catchers greatly increases their efficiency. Jack (1941) had his group carry a dark grey blanket (1.50 × 0.90 m) stretched on a pole. Macaulay (1942) and Barrass (1960) used the same method, whereas Chorley (in Buxton 1955) placed the screen on a bicycle.

Vale (1969, 1972) described various mobile screens: a black drum mounted on wheels, screens covered with glue and carried on human backs or placed on a bicycle or other vehicle, electrified screens hauled behind mobile bait, and electrified cages containing bait. The radius of action of screens varies according to species. For *G. medicorum* it is only 8 m, whereas for *G. morsitans* it reaches 50 m (Chapman 1961).

Mobile screens have the major disadvantage of requiring carriers. Immobile screens require the presence of catchers but one operator may operate several traps.

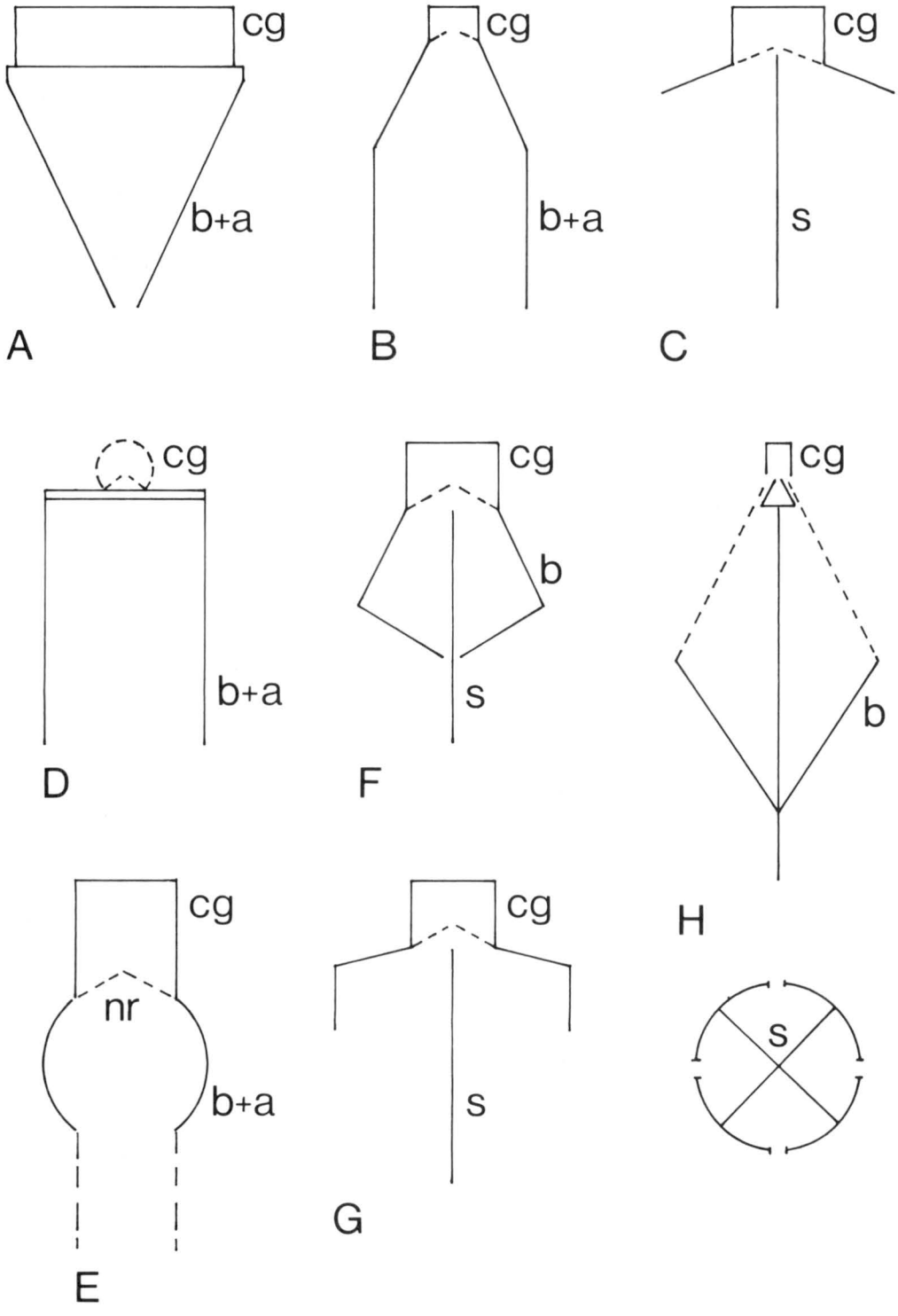
Falling Cages and Artificial Refuges

Man-Activated Mobile Bait Trap of Goiny (1967)

The device is activated by a man who drops it around himself at regular intervals. Captive *Glossina* move upwards into a cage exposed to light at the top of the trap.

Falling Cage of Phelps (1968)

The falling cage is a structure made of mosquito netting mounted on an iron frame (2.6 × 2.1 × 1.8 m). With the aid of a pulley, it is lowered every 10 minutes. In the upper position it stands at 2 m above the ground, enabling tsetse flies to come close to the bait that is placed under the trap.



Schematic cross sections through various models of traps: A Harris' trap; B Chorley's trap; C Swynnerton's AS trap; D Lewillon's dummy; E trap of Morris and Morris; F. Langridge's trap; G Moloo's trap; and H biconical trap of Challier and Laveissière, nr = no-return device; b = body; b + a = body with attracting surface; cg = cage; s = attracting screen.

Artificial Refuges of Vale (1971)

To study the behaviour of *G. pallidipes* and of *G. morsitans* at rest, Vale built various types of artificial refuges: box, hut, and trellis. He also used cement pipes. Tsetse flies coming to these refuges are caught with a net, although Vale added to some of his models a mechanical catching device.

As is the case with screens, Goiny traps, and falling cages, artificial refuges have the disadvantage of requiring catching personnel.

Traps Proper

Principle of Tsetse Trapping

Trapping is based on the tendancy of adult *Glossina* spp. to move towards dark surfaces and shaded cavities, the body of the trap being mistaken for the belly of an animal (Fuller and Mossop 1929). Once in the dark cavity, the tsetse flies find themselves in an overly dark environment. They are then attracted towards the lighted summit of the trap. Urged to climb ever higher, they pass the "no-return device" and enter the cage.

General Description of a Trap

A tsetse trap includes essentially the following elements: (1) a body, i.e. a dark enclosure of varied shape; (2) an attracting surface, which may constitute the wall of the body itself or be an independent element placed inside or under the body; (3) a collecting cage placed at the top of the trap, exposed to light; (4) a "no-return device" having the shape of a pyramid or slot located between two planes in an inverted "V" converging in a cage; (5) auxiliary equipment — supports (pole driven in the ground, posts and suspension ropes, legs) and an antipredator device; and (6) an attractant, i.e. bait animal, substances extracted from animals, chemical compounds, or physical attractant such as sound.

Models of Traps

Harris' Trap

This, the first trap specifically designed for *Glossina* (Harris 1930), enjoyed real success in Zululand in catching *G. pallidipes*. The final model ("standard trap," Harris 1938) measured 1.83 m in length and 0.91 m in height.

The Harris trap was used in Zaïre (Merken 1934; Lewillon 1945), in Tanzania (Swynnerton 1936), and in Botswana (Macaulay 1942).

Chorley's "Crinoline"

According to Swynnerton (1936), Chorley's (1933) "crinoline" was constructed on the same principle as the trap developed by Richmond and Mendis in India to capture mosquitoes. This trap consists of a cylindrical "skirt" made of jute that is mounted on metal rings and has an opening at the base. The cone-shaped top of the trap gives access to a small cage.

Swynnerton's Traps

Swynnerton (1933, 1936) described several screens that are, in fact, true traps: plain screen trap or PS trap, awning screen trap or AS trap, etc. Some of these models are mechanized or contain a bait animal. Swynnerton's sustained efforts to develop a high-yield trap did not result in the general availability of a simple and efficient model.

Jack's Trap

Glossinids enter this trap (Model No. VIII, Jack 1939) from the shady side as well as from the bottom. The "tin-top" (Jack 1941) is a modification of Harris' trap.

Lewillon's "Dummy"

The "dummy" is a vertical parallelepiped device of black raffia, 1 m in height, and suspended from a horizontal square board. In the centre of the board a square hole is cut to give access to a no-return de-

Langridge trap in use, catching G. pallidipes in maize field, Lambwe Valley, Kenya (WHO photo, D. Baldry).

vice with fine mesh that is topped by a water-bottle with holes, which serves as a cage. A frame keeps the skirt open.

Animal Trap of Morris and Morris

Initially, this trap simulated a goat as to size, skin, head, and legs; however, its realism was seemingly appreciated more by the entomologists than by the tsetse (Morris and Morris 1949).

The "standard model" is a simplified version of the original one (60 × 18 × 93 cm in height). It consists of a body mounted on four legs and is made of rough fabric supported by a semicircular iron frame. On the upper opening rests a parallelepipedal cage equipped with a no-return device made of two planes disposed in a "V." On one of the lateral surfaces of the cage there is a sleeve for removal of the flies.

Morris (1961a) built a model with dimensions double those of the "standard" one as well as a portable model, which could be taken to pieces.

Langridge's Trap

Langridge's trap (1968) is an adaptation of the Harris trap. It includes a vertical screen (90 × 86 cm), a dark or black cotton and wool blanket that has a 10-cm wide hole into the "body." The latter is a box with inclined surfaces, the two upper ones being of green wire meshing and the two lower ones of black fabric. A wire-mesh cage, cream in colour and equipped with a no-return device, is fitted at the upper opening of the body.

Moloo's Trap

Moloo's (1973) model was derived from Langridge's. It differs from it by having an awning, extending downward into a "skirt," made from a black blanket and by eliminating the lower slit of the body. This

new device enables the flies to be attracted to a larger shady space than that of the preceding model.

Bioconical Trap (Challier and Laveissière)

In this model, the trapping principle consists in attracting the glossinids to the "body" by means of screens placed inside it (Challier and Laveissière 1973).

Two cones joined together at the base (80 cm diameter and 133 cm total height) make up the body. Mosquito netting encloses the upper cone, the lower being of white percale with four vertical openings. Screens, arranged in a cruciform fashion inside, rise to two-thirds of the height of the upper cone. This structure is supported by an apical pyramidal frame of metal, the summit of which penetrates a cage of the Roubaud type. The base is fitted into a metal tube that supports the trap.

Behaviour of Glossinids and Attraction to Traps

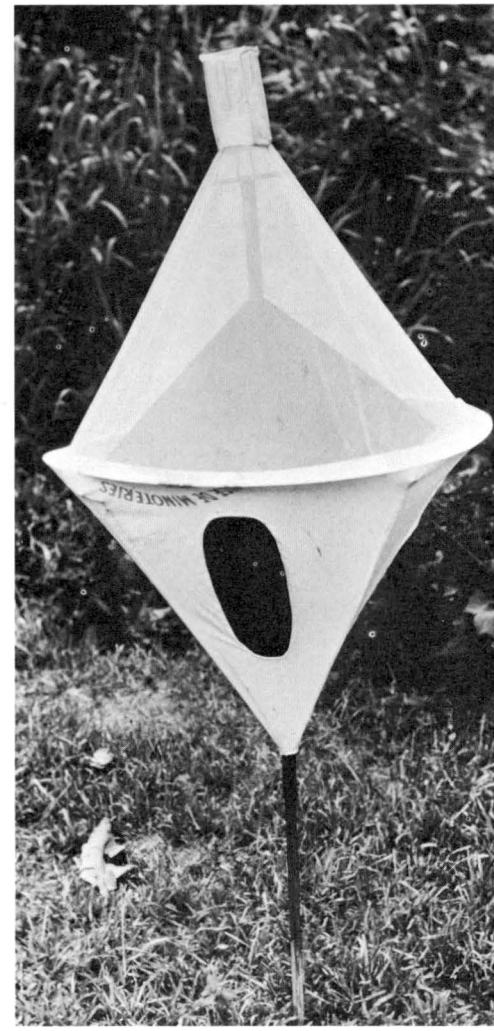
It is believed that adult *Glossina* come to traps when hungry (Swynnerton 1933). Their reaction would then be that of an insect in search of its host (Lloyd 1940; Saunders 1964a), and the need for a blood meal would overcome the necessity of finding shade (Morris and Morris 1949). However, according to Jack (1939, 1941), hunger is not the primary factor — he stated that the flies are attracted to the points of greatest light intensities in the biotope.

To locate a trap an insect must be in its active phase. In the laboratory, tsetse flies show a daily V-shaped cycle of spontaneous activity. The threshold of response to stimuli increases in relation to the fasting stage (Brady 1972b, 1973). There is a correlation between spontaneous activity and the weight of the insect, as well as the amount of residual blood. Fasting could therefore produce the sensory impulse needed to trigger the spontaneous activity.

Tsetse react positively to light (Mellanby 1936). Above 30 °C, though, phototropism becomes negative whatever the temperature may be in the shade (Jack and Williams 1937). In nature they move in and out of shaded and lighted zones but remain inactive during the hottest period of the day. Biting behaviour is conditioned mainly by visual and olfactory stimuli.

Tsetse, perceiving rapid and gentle movements, are stimulated by moving

Biconical trap in operation in gallery forest near village of Tin, Upper Volta (A. Challier).



Blue-coloured cylindrical trap in experimental field use at Nasso, near Bobo-Dioulasso, Upper Volta, G. palpalis habitat, 3 May 1974 (M. Laird).

screens (Chapman 1961). The shape of an object and its contrast with the background are important factors in the detection of hosts (Turner and Invest 1973). Teneral[13] individuals, and males and females react differently to stimuli (Brady 1972a). The "visual response" increases in the presence of olfactory stimuli. Teneral males of *G. morsitans* are more sensitive to moving objects than females, which respond better to objects displaying interrupted movements (Gatehouse 1972).

It has long been known that dark colours attract *Glossina* more than light ones. This applies to screens (Maldonado 1910; Lloyd 1935; Swynnerton 1936; Barrass 1960, 1970; Chapman 1961) as well as to animals (Moggridge 1949; Morris 1960; Harley 1963; Smith and Rennison 1961a; Saunders 1964a). However, Jack (1939) noticed a dark blue screen attracted *G. morsitans* better than a donkey.

In the laboratory, tsetse flies show colour preferences in the following decreasing order: ultraviolet, blue, red, white, and yellow. In a group of colours, gray is more attractive if the colours are separated by light patches (Dean et al. 1969a).

In nature, light conditions may change the order of colour preferences. Thus in Botswana, *G. morsitans* adults were attracted by boards suspended from branches in the following decreasing order: white, yellow, green and black, blue, and red in the case of one-colour boards; and red-blue, white-black, red-black, black-yellow, blue-yellow, and red-green in the case of two-colour boards (Lambrecht 1973). The strong attraction of the white board is due to its contrast with the environment.

Tsetse flies perceive odours from a distance. *G. swynnertoni* can locate a hidden crew of catchers and oxen at a distance of nearly 55 m (Bax 1937). According to Dean et al. (1969b), vision would play a role at long distances and smell at short distances,

[13] The teneral state is the condition of tsetse that have not yet taken their first blood meal and still have a soft integument (ed).

but the relative importance of these two senses varies from species to species.

Trap Efficiency

Yield Factors

The yield factors of a trap are: (1) intrinsic factors of the trap; (2) use of attractants; (3) environmental factors; and (4) factors associated with methodology.

Intrinsic Factors of the Trap

The use of screens requires the presence of catchers and sometimes of bearers. An

Painted fly-traps determine which colour attracts the most insects (WHO photo, J. Abcede).

Table 10. Capture of *G. p. gambiensis* with various traps. Yield per trap per day (Y_{pd}); comparative yields of various methods of capture, taking as unit the results obtained with other types of traps, a catcher (c), and ox (o) as bait (Y_t/t, o, c); sex-ratio (ref: method used as unit, p.s. ext. = pig skin extract).

Locality	Month	Type of trap or other means of capture	Y_{pd}	Y_t/t, o, c male	Y_t/t, o, c female	Sex ratio (% females) Trap	Sex ratio (% females) Other	Authors
GHANA	Jan-Dec	Morris (animal trap)	3.1	0.07 (c)	0.09	52	49 (c)	Morris & Morris 1949
Volta R.		Morris (animal trap)		1 (ref)				
		Harris		0.01				
		Swynnerton (ASB)						
		open vegetation		0.05				
		woods		0.43				
		Chorley (crinoline)						
		open vegetation		0.06				
		woods		0.21				
LIBERIA	Nov–Dec	Morris (animal trap)						
Lawa R.		standard	8.7			67		
	Jan–Mar	double-size	9.8			60		
		Morris (animal trap)						
		standard	1.85			61		
		double size	2.85			53		Morris 1961a
		Morris (collapsible)						
		drill		0.20				
		jute		1 (ref)				
Zeliba R.		Morris (standard)		1.38 (c)		76.6	65.4 (c)	
IVORY COAST Léraba R.	Mar–July	Challier-Laveissière (biconical trap)	13.1	0.64(c)	1.29	69.5	53.0(c)	Challier & Laveissière 1973
UPPER VOLTA Kou forest	Feb–Mar	Challier-Laveissière (biconical trap)	15.3	0.28(c)	1.24	67.2	37.3(c)	

adhesive obviates this inconvenience, but glued specimens are hardly in ideal condition.

The best traps are those that provide a shaded area in such a way that tsetse, attracted from afar, may mount into the lighted part of the trap. Tables 10–14 show the results of captures with various models.

Large traps are more evident from a distance than small ones. Morris (1960) caught three times as many specimens of *G. pallidipes* with a trap twice the size of his "standard trap." This large trap, however, had no success in catching *G. palpalis* and *G. tachinoides*. A model set on higher legs did not improve the yield (Morris 1961a).

The attraction of *Glossina* spp. by colours is a complex phenomenon that has not yet been satisfactorily explained. Darker colours are not necessarily the most attractive. *G. pallidipes* come in larger numbers to a brown Morris trap than to a black or dark gray one (Morris 1960; Saunders 1964a). The same trap is more efficient when it is covered with natural jute than with jute painted black (Smith and Rennison 1961a), and khaki drill is as efficient as black (Morris and Morris 1949). The biconical trap attracts by the strong contrast of its shape against a background of dark vegetation and by the black screens visible through the opening of the white surface (Challier and Laveissière 1973).

Table 11. Capture of *G. f. fuscipes* with various traps. Yield per trap per day (Y_{pd}); comparative yields of various methods of capture, taking as unit the results obtained with other types of traps, a catcher (c), and ox (o) as bait (Y_t/t, o, c); sex-ratio (ref: method used as unit, p.s. ext. = pig skin extract).

Locality	Month	Type of trap or other means of capture	Y_{pd}	Y_t/t,o,c, male female	Sex ratio (% females) Trap	Other	Authors
TANZANIA	May	Harris	3.5				Swynnerton 1936
Kuja R.		Swynnerton (SS)	40.5				
ZAIRE		Lewillon (mannequin)	0.23				
Kwange		Harris	0.18				Lewillon 1945
		Swynnerton (SS)	0.30				
UGANDA		Morris (standard)					
Busoga		normal	6.8–3.9				
		+p.s. ext.	8–7.3		50		Persoons 1965b
		Langridge					
		normal	6.5–3				
		+p.s. ext.	8.8–4.4				
UGANDA		Morris					
		normal		1 (ref) 1			Rogers 1969
		+dry ice		4.7 5.1			
UGANDA	June–July	Swynnerton (AS)	7.6		53.5		
Busoga		Langridge (BS)	33.1		55.4		
	Aug–Oct	Langridge (BS)	8.4		46.5		Moloo 1973
		Moloo (ASS)	53.4		62.6		
	Oct–April	Swynnerton (AS)	0.75		72.0		
		Langridge (BS)	14.6		81.9		
		Moloo (ASS)	56.7		77.3		

Table 12. Capture of *G. tachinoides* with various traps. Yield per trap per day (Y_{pd}); comparative yields of various methods of capture, taking as unit the results obtained with other types of traps, a catcher (c), and ox (o) as bait (Y_t/t, o, c); sex-ratio (ref: method used as unit, p.s. ext. = pig skin extract).

Locality	Month	Type of trap or other means of capture	Y_{pd}	Y_t/t, o, c, male female	Sex ratio (% females) Trap	Other	Authors
GHANA	Jan–Dec.	Morris (standard)					
(north)		Volta R.	32.7	0.30(c)0.11	35	61(c)	
		woods	6.0	0.22(c)0.23	45	43(c)	
		Morris (standard)		1 (ref)			
		Harris		0.04			Morris & Morris
		Swynnerton (ASB)					1949
		open vegetation		0.12			
		woods		0.41			
		Chorley (crinoline)					
		open vegetation		0.16			
		woods		0.12			
IVORY COAST	Mar–July	Challier-Laveissière	157.9	0.64(c)1.63	65.1	42.3(c)	Challier &
Léraba R.		(biconical trap)					Laveissière 1973

Table 13. Capture of *G. pallidipes* with various traps. Yield per trap per day (Y_{pd}); comparative yields of various methods of capture, taking as unit the results obtained with other types of traps, a catcher (c), and ox (o) as bait (Y_t/t, o, c); sex-ratio (ref: method used as unit, p.s. ext. =pig skin extract).

Locality	Month	Type of trap or other means of capture	Y_{pd}	Y_t/t,o,c, male	female	Sex ratio (% females) Trap	Other	Authors
TANZANIA Shinyanga	Sept	Harris	41			58.7		
		Swynnerton						Swynnerton 1936
		JV	49.3			76.1		
		AS	51.0			76.5		
UGANDA Busoga	Mar–Dec and Jan-June	Morris (standard)						
		brown	1.9			84.7		Morris 1960
		black	2.7			82.3		
KENYA Lambwe Valley	Dec	Morris (standard)	17.4	4.40(c)		78	17(c)	Glasgow & Duffy 1961
UGANDA Busoga	July–Sept	Morris (standard						
		brown	46	0.14(b)	0.62	77.3	44.3(b)	
		brown	24	0.15(b)	0.69	75.9	40.2(b)	Saunders 1964a
		black	11	0.10(b)	0.27	65.9	40.2(b)	
UGANDA Busoga		Morris (standard)						
		normal	114.5–58.5			70–80		
		p.s. ext.	139.5–103					Persoons 1965b
		Langridge						
		normal	116.5–87.5					
		ext. p.p.	202–183.5					
UGANDA Busoga		Morris (standard)						
		normal		1 (ref)	1			Rogers 1969
		p.s. ext.		5.3	2.3			
KENYA Lambwe Valley	Dec–Jan	Langridge	258	4.36(c)		63.9	18.9(c)	
		Man		1 (ref)				
		Calf		4.23				England & Baldry 1972
		Langridge trap		1.42				
		Sheep		0.66				
		Goat		0.51				
UGANDA Busoga	June–July	Swynnerton (AS)	0.4			62.9		
		Langridge (BS)	39.1			76.9		
		Langridge (BS)	26.5			77.4		Moloo 1973
		Moloo (ASS)	56.8			79.4		
		Swynnerton (AS)	0.75			72.0		
		Langridge (BS)	14.6			81.9		
		Moloo (ASS)	56.7			77.3		

Use of Attractants

Tsetse flies move toward a trap which, once sighted, attracts them by its shape and its colour. Morris (1960) estimated the radius of action of a trap as 5–10 m. To improve its efficiency, it is therefore important to add an attractant. Olfactory attractants include bait animals, organic substances extracted from animals, and chemical compounds (Hughes 1957a).

The presence of an animal, even when hidden, increases the number of flies caught (Lloyd 1935; Swynnerton 1936). A

Table 14. Capture of *G. m. submorsitans*, *G. longipalpis*, and *G. nigrofusca* with various traps. Yield per trap per day (Y_{pd}); comparative yields of various methods of capture, taking as unit the results obtained with other types of traps, a catcher (c), and ox (o) as bait (Y_t/t, o, c); sex-ratio (ref: method used as unit, p.s. ext. =pig skin extract).

Species	Locality	Month	Type of trap or other means of capture	Y_{pd}	Y_t/t, o, c, male	Y_t/t, o, c, female	Sex ratio (% females) Trap	Sex ratio (% females) Other	Authors
G. m. submorsitans	GHANA Kamba R. Bridge	Jan–Dec	Morris (standard)	3.4	0.08(c)	0.10	46	40(c)	Morris & Morris 1949
G. longipalpis	GHANA Cape Coast	Oct–Nov	Morris (standard)	1.5 (male) 2.4 (female)	0.43 (c)		60.1		Morris 1961b
G. nigrofusca	GHANA Kumasi	April–Aug	Morris (standard)	0.13			50		Morris 1961b

screen placed in contact with a bush pig or treated with extracts of this animal is more attractive than a nontreated screen (Vanderplank 1944). Cow urine and dung attract *G. pallidipes*. *G. medicorum* reacts to cow odour and to acetic acid fumes (Chapman 1961), whereas exhaust gases from automobiles and crank-case oil distillates have practically no effect on *G. palpalis* (Hughes 1957b).

Experiments made with an olfactometre on fresh citrated ox and rabbit blood, on CO_2, on L-lysine, and on alcoholic extracts of pig skin showed that the study of tsetse behaviour in the laboratory is difficult (Persoons 1965a), as had already been reported by other authors (Chapman 1961; Hughes 1957a, b).

Field experiments give more definitive results than laboratory ones. Langridge (1961) doubled the number of *G. pallidipes* caught by treating paths with lanolin and quintupled them with extracts derived from pig skin, hairs, and scrapings. The same extracts attracted from two to three times more examples of *G. austeni* with a residual effect, which persisted for nearly 3 weeks (Anonymous 1962). Wool-fat extracts are more efficient when used in the raw state than when the purified products are used (MacOwen 1959). Park (1965) showed that the most attractive compounds, extracted from organic products taken from an abattoir, are those soluble in a fat solvent.

Langridge and Morris traps, treated twice a week with pig skin extracts soluble in benzene and petroleum ether, caught twice as many *G. pallidipes* specimens as control traps (Persoons 1965b, 1966, 1967). The yield may be increased by a factor of 2.7.

At a concentration of 0.01%, L-lysine had, at first, a repellent effect on *G. pallidipes* and *G. f. fuscipes*. One week after treatment, though, the yield increased 4.22 times for the first species and 1.81 times for the second, during a period of 3 weeks (Persoons 1968).

The first trials with CO_2 in a Morris trap failed (Rennison and Robertson 1959; Maudlin 1969), but Rogers (1969) obtained a fivefold increase by using dry ice.

Harley (1965b) modified the Morris trap somewhat by placing a metal plate under the jute to heat the attractant by solar rays; results were negative. It would appear that a high temperature inside the trap does not suit tsetse flies.

Maudlin (1969), Dean et al. (1969a), and Turner (1971) unsuccessfully attempted to find a sexual pheromone secretion that would attract males, but Langley (1975) reported that it had been demonstrated at Langford that a male *G. m. morsitans* will mate with a dead female but not with a male. The attractiveness of dead flies to sexually mature males is progressively reduced by washing the former in various solvents, and is abolished by nonpolar solvents (Langley and Pimley 1975). Decoys (dead males of their species, or "pseudo-

flies" made from black shoelace knots) were also both found unattractive to sexually mature *G. m. morsitans*. However, when such decoys were shaken with female *G. m. morsitans* in diethyl ether or hexane, and the solvent was allowed to evaporate, they became highly attractive to sexually mature males and maintained this attractiveness for at least 4 days. The implication was "that a sex recognition pherome of lower vapour pressure than the solvents used had been transferred from the female flies to the decoys." More recently, Nash et al. (1976) have reported the presence, in the same species, of a larval pheromone that may attract gravid females to a larviposition site.

Sound could be used as an attractant since tsetse react to certain frequencies (Langridge 1964; Kolbe 1972).

Environmental Factors

The efficiency of a trap depends on the ease with which it is detected by *Glossina*. The location of the trap in the biotope and the components of the environment influencing insect behaviour are therefore important factors.

The traps must be located in optimal light and shade conditions (Harris 1938). For capturing *G. palpalis*, Morris and Morris (1949) placed their trap on "hunting grounds" (fords, bridge approaches, water holes, drinking and washing places, paths, etc.). As a general rule, borders of forest galleries and of thickets are chosen (Morris and Morris 1949; Glasgow and Duffy 1961; Saunders 1964a). Challier and Laveissière (1973) observed that the number of tsetse caught increases when traps are located at more open and even sunny locations.

Optimal siting of traps in relation to the sun and to the biotope is an important condition required for a good yield. The maximum number of *G. pallidipes* is caught in the afternoon on the eastern border of a thicket, and before noon on the western border (Glasgow and Duffy 1961). In western Africa, *G. p. gambiensis* tend to emerge from the eastern end of forest galleries at sunrise (Challier et al. 1974). The trap must therefore be sited in such a way that all day long it makes a marked contrast with its environment.

The number of tsetse attracted may be increased if a man or an animal is in the vicinity of the trap (Swynnerton 1933; Symes and Southby 1938; Macaulay 1942; Morris 1960); vehicles are also an attractant (Chorley 1947).

Swynnerton (1933) is of the opinion that the best yield from traps is obtained during the hot period of the year. In western Africa, the captures of *G. tachinoides* are maximal in January (dry season) and minimal in September (Morris and Morris 1949). Jack (1939) reported negligible captures after the onset of the rainy season. According to Jack (1944), the evaporation rate would therefore be an important factor. Drought produces a loss of water in the fly, which reacts by searching for shade. During the dry season, shade is reduced to a minimum and, as a result, the flies are attracted by the traps. Morris and Morris (1949) did not accept this explanation for *G. palpalis*. During the rainy seasons, the catchers, crisscrossing biotopes in which populations are dispersed, capture more tsetse than do the traps. Moreover, at that time, visibility is less than during the dry season, whereas people visit the water holes less frequently.

Factors Associated with Methodology

A good trap must be simple, strong, light, and able to be taken to pieces so that it occupies little space during transport. It must also be easy to assemble rapidly. These qualities enable the traps to be used in large numbers by a minimum number of personnel.

A certain number of organizational problems in trapping have been studied. Harris (1938) found that an increase in the number of traps in a biotope caused a decrease in the yield of the units already in operation. For a certain period of time, the latter catch more flies than the newly installed traps, undoubtedly because these flies spread out more slowly.

The numbers caught by a Morris trap

are not affected by an emptying rhythm varying from 1.5 to 24 h. Beyond the longer interval, there are mortalities due to predators (particularly ants). An entrance slot of 12 mm gives the same results as a 38 mm one.

Escapes become important when the number of 12-mm-square holes in the cages reaches 16, and especially when the catchers approach the trap (Rennison and Smith 1961).

Trap Yields

The various models of traps do not have the same efficiency with different species of *Glossina*. *G. brevipalpis* is hardly, if at all, attracted by traps (Jack 1941; Saunders 1964a; Moloo 1973). Morris (1961b) was able to capture specimens of *G. pallicera* and of *G. medicorum*, but none of *G. nigrofusca*. *G. p. gambiensis* comes to a trap, but less readily than *G. tachinoides* (Morris and Morris 1949; Challier and Laveissière 1973).

Some authors have compared the yield of their models of traps with that of their predecessors and of other methods of capture (see Tables 10–14 for examples). According to Morris (1960) the efficiency of a crew of catchers is comparable to that of one man supervising eight traps.

A good way of comparing the efficiency of traps with that of catchers consists in permuting the elements at various sites of capture. The yield may then be expressed in numbers of tsetse caught in a trap for every 100 of these insects caught by catchers (Morris and Morris 1949), or in numbers of tsetse/day/trap. Tables 10–14 give the yield of various models calculated from published data whenever the authors gave sufficient information for uniform expression of their results.

Quality of Sampling

Qualitative Aspects

Sex-Ratio

Traps generally catch a higher proportion of females than other methods of capture. The number of females is almost always higher than that of males (Tables 9–13).

Sex-ratios vary with trap location and season. In the case of *G. tachinoides*, it is lower during the dry than during the rainy season (Morris and Morris 1949). Challier and Laveissière (1973) observed that the more the trapping location was open and sunlit, the more *G. p. gambiensis* they caught.

Feeding State

Jack (1941) pointed out that in haematophagous insects the positive reaction to traps was associated with biting behaviour. Thus, both sexes of *Stomoxys* and *Glossina* come to the traps because the two are haematophagous, whereas this behaviour is characteristic of females only in tabanids.

In *G. pallidipes* samples caught with traps, the proportion of starved males is lower than in those caught on oxen (Smith and Rennison 1961b, c), but females are never very hungry because they possess reserves of fat (Jack 1941).

Individuals that come to the traps include: (1) for *G. palpalis* and *G. m. submorsitans* (Morris and Morris 1949) as well as *G. f. fuscipes* (*G. palpalis* auct. Lloyd 1940), both sexes when they are hungry; (2) for *G. pallidipes* and *G. morsitans*, females at all alimentary stages and males when they are hungry — this is also the case for *G. brevipalpis*, but there is some uncertainty concerning the males (Jack 1939); and (3) for *G. tachinoides*, in addition to starved individuals, there are also males seeking females (Morris and Morris 1949).

Age Composition of Samples

Traps catch a higher proportion of older females than any other method. The *G. pallidipes* females caught in a Morris trap are older than those caught on men, on moving or stationary vehicles (Harley 1967), or on a zebu or an ox (Saunders 1962, 1964a).

In the case of *G. f. fuscipes*, the relationship with age is not as close as in the above species (Harley 1967; Saunders 1962). In the case of *G. tachinoides*, however, the proportions of tenerals and old parous flies in the trap samples are the reverse of those caught by the catchers (Challier and Laveissière 1973). This situation, therefore, has a direct influence on the rate of tsetse infection by trypanosomes, since the proportion of infected flies is higher among the old than among the young (Saunders 1962; Harley 1967).

It would appear that the behaviour of *Glossina* changes with age (Saunders 1962). Brady (1972a) observed in the laboratory that the effect of olfactory stimuli added to that of visual stimuli is stronger in teneral males than in the older ones. In the field, the proportion of young *G. f. fuscipes* increases when the trap contains dry ice (Rogers 1969).

Pregnancy

Gravid females come more readily to traps than to catchers. They mistake traps for larviposition sites (Saunders 1962, 1964a).

Quantitative Aspects

Traps, like other methods of capture, do not give satisfactory quantitative results because daily fluctuations in numbers caught are too large. The longterm fluctuations shown by trapping follow those observed by classical methods, but are not significantly correlated (Glasgow and Duffy 1961). However, Tarimo et al. (1970) were of the opinion that trapping was the best method for assessing the decline of *G. pallidipes* populations after aerial applications of insecticide.

When trapping extends over a long period, the first captures are the largest. After a decline, the captures remain more or less at the same level. There is a "skimming" of starved individuals (Morris and Morris 1949; Morris 1960). The shape of the activity curve established from trapping differs somewhat from the one based on capture by nets or other methods (Glasgow and Duffy 1961; Smith and Rennison 1961b). Glasgow (1956) raised the question of trap interactions when these are used in large numbers.

The Use of Traps

Advantages

According to Morris (1961b), traps have the following advantages over classical methods of capture: (1) possibility of uniformization of data when a large number of observations is required, i.e. more traps may be added; (2) elimination of negative elements of the capture with nets, such as fatigue, absence due to sickness or bad weather; (3) economy of personnel, i.e. one man may supervise each day about 20 traps scattered over several kilometres; (4) the traps may remain in one locality for 24 h, thereby functioning during the whole activity period of the glossinids, whereas mobile crews may miss the activity peak during that period; and (5) variations in numbers caught reflect changes in the environment or in the behaviour of flies.

As a Research Tool in Ecology

Traps are useful for the study of all aspects of ecology (e.g. activity, dispersal, ecodistribution, and population dynamics).

The study of dispersal is facilitated by the possibility of placing a large number of traps over vast areas, but the fact that the radius of action of traps is only about 10 m (Glasgow and Duffy 1961) must be taken into account.

Investigators who have used traps to recapture specimens that had been marked and released have not been altogether satisfied with the results, because recapture rates have been low (Rennison and Smith 1959; Vale 1971). However, Symes and Vane (1937) obtained 12% recaptures.

Traps are an economical complementary method in surveys conducted to ascertain the distribution of species or their rates of infection. It is possible with them, too, to multiply the points of capture without increasing the numbers of catchers. Moreover, they are better suited to detect low densities than more usual methods (Glasgow and Duffy 1961; Glover and Langridge 1963).

As a Control Method

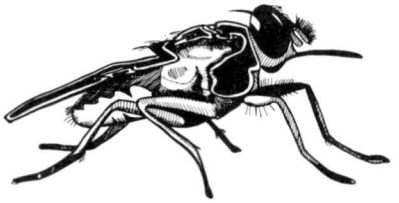
The first tsetse control operation was started on Principe Island with *G. palpalis*, using screens covered with glue (Maldonado 1910). A few years later, da Costa et al. (1916) adopted, as a technique, forest clearing and animal killings. However, they continued using black screens on the backs of men dressed in white. In this manner, Principe was rid of *G. palpalis*.

Harris traps were used in Zululand against *G. pallidipes*. In 1931, 487 traps captured 7 million flies, whereas in 1937, 8928 traps caught only 57 000 (Harris 1938). In Zaïre, Lewillon (1945) used his "dummy" in sleeping sickness areas. Morris (1961b), in Ghana, protected a temporary hospital and a college by placing his "animal-trap" at the edge of the forest.

After the reinvasion of Principe Island by *G. palpalis*, 4651 Morris traps treated with DDT were used to reinforce other methods of control (Azevedo et al. 1962; Azevedo and Pinhão 1969). Morris (1950) recommended the use of 50 traps/ha impregnated with DDT at a concentration of 47–80 g/m^2 for control purposes. Rupp (1952), facing the problem of residual populations after aerial insecticide sprayings, impregnated attracting fabrics with DDT.

Traps are not of themselves sufficient to eradicate tsetse flies (Van Hoof et al. 1938; Glover and Langridge 1963). With them, however, it is possible to obtain marked local reductions of vectors (Morris and Morris 1949).

Goiny (1967), who was able to catch 440 tsetse/hour in a heavily infected area, was of the opinion that 20 of his traps could rid nearly 1300 km of gallery forest, stream banks per year of their tsetse flies. Perhaps the new knowledge on tsetse attractants, including pheromones, will open up new ways to make traps more effective as research tools and for practical control purposes. — A. Challier.



bility of establishing tsetse colonies. Unsuccessful pioneer laboratory breeding attempts (with references additional to those herein), and successful efforts in the 1950s and 1960s have already been reviewed by Azevedo (1970). Some of these original colonies have, for various reasons, ceased to exist (Table 15). Only five laboratories now remain where tsetse production is proceeding at such a level that several thousand adult flies are always on hand, without external input and according to standardized techniques.

Mass Rearing Using Animals for Feeding

As early as the beginning of this century, in the early days of European scientific activities in Africa, a few experimenters attempted to rear small numbers of tsetse flies. Most of these workers were able to keep only a few flies alive during a limited period (Roubaud 1917a). Promising results were, however, achieved by some, albeit on a limited scale (Willett 1953). Later, other investigators established in Africa more important rearings, which enabled them to obtain necessary research material. However, none of these rearings were really autonomous, since the reinforcement of colonies was generally assured from insects caught in the field (Nash et al. 1958).

After the 1950s, fundamental research on African trypanosomiases and their vectors was intensified in Europe and North America. This resulted in an increased demand for large numbers of live tsetse flies. At the time, it was not known whether or not it was possible to create self-maintaining tsetse colonies to satisfy the research needs, although results obtained by Geigy (1948) encouraged the belief that this goal could be attained.

Experiments were carried out in various European countries to assess the possi-

Colonies in Africa

In Africa, it is often difficult in the laboratory to: (1) maintain temperature and humidity conditions needed by *Glossina* spp.; (2) provide effective protection against predators (ants, cockroaches, earwigs, etc.); (3) avoid infections by bacteria or fungi; and (4) insure protection against accidental intoxications by various chemical substances, especially insecticides. Therefore, there have been few tsetse colonies established with lasting success, and these have only been maintained with great difficulty. In the 1950s, however, Nash et al. (1958) succeeded in maintaining a significant level of laboratory production of *G. palpalis* at Kaduna, Nigeria. This, however, never became totally self-sustaining — that is, there had to be intermittent supplementation of the stock from fresh field collections.

From 1947 to 1954, Evens (1954) maintained *G. palpalis* (*G. f. quanzensis*) in the laboratory at Kinshasa (formerly Leopoldville). In the absence of equipment necessary for the maintenance of correct climatic conditions, the life span of the females did not exceed 22 days and it was not possible to achieve a self-maintaining colony.

In Tanzania, Willett (1953) was able to raise *G. swynnertoni* to the eighth generation, but a prolonged dry season caused the colony's decline during the following

Table 15. Tsetse colonies in Europe, 1959–1974.

Authors	Laboratories	Start	Species	Origin	Present situation
Azevedo et al.	Escola Nacional de Saude Publica e de Medicina Tropical, Lisbon, Portugal	Sept. 1959	*G. m. morsitans*	Mozambique	Colony extinct about 1971 (intoxication by insecticide)
Nash and Jordan	Tsetse Research Laboratory Langford, Bristol, U.K.	June 1963–April 1966	*G. austeni*	Tanzania	Autonomous colony in stationary phase
Itard and Maillot	Institut d'Elevage et de Médecine Vétérinaire des Pays Tropicaux, Maisons-Alfort, France	August 1964	*G. m. centralis*	Tanzania	Rearing stopped end 1971
Itard and Maillot	IEMVT, Maisons-Alfort, France	Feb–July 1965	*G. m. morsitans*	Rhodesia	Autonomous colony in stationary phase
Azevedo and Pinhão	ENSPMT, Lisbon, Portugal	April 1965	*G. m. submorsitans*	Nigeria	Colony extinct about 1971 (intoxication by insecticide)
Itard et al.	IEMVT, Maisons-Alfort, France	April 1965–Dec 1966	*G. tachinoides*	Chad	Autonomous colony in stationary phase
Azevedo and Pinhão	ENSPMT, Lisbon, Portugal	July 1966	*G. m. morsitans*	Rhodesia	Colony extinct about 1971 (intoxication by insecticide)
Itard	IEMVT, Maisons-Alfort, France	Nov 1966–Jan 1967	*G. austeni*	Langford (Bristol-U.K.)	Autonomous colony in stationary phase
Jordan et al.	TRL Langford, Bristol, U.K.	Feb–Mar 1967	*G. m. morsitans*	Rhodesia	Autonomous colony in stationary phase
Evens and van der Vloedt	Laboratorium voor Oekologie, Antwerp, Belgium	June 1967	*G. m. morsitans*	Rhodesia	Rearing stopped end 1970
Evens and van der Vloedt	LVO, Antwerp, Belgium	Feb 1968	*G. p. palpalis*	Zaïre	Autonomous colony in stationary phase
Evens and van der Vloedt	LVO, Antwerp, Belgium	April 1968	*G. f. quanzensis*	Congo	Autonomous colony in stationary phase
Langley et al.	Seibersdorf Laboratory (IAEA), Vienna, Austria	end of 1968	*G. austeni*	Langford (Bristol, U.K.)	Rearing stopped 2nd quarter 1970
Itard	IEMVT, Maisons-Alfort, France	June 1968–Jan 1969	*G. f. fuscipes*	Central African Rep.	Autonomous colony in stationary phase
Langley et al.	IAEA, Vienna, Austria	Begin. 1969	*G. m. morsitans*	Lisbon (Portugal) +Rhodesia	Autonomous colony in stationary phase
Pinhão	Instituto de Higiene et Medicina Tropical, Lisbon, Portugal	Feb 1971	*G. m. submorsitans*	Nigeria	Autonomous colony in stationary phase
Evens and van der Vloedt	LVO, Antwerp, Belgium	Jan 1972	*G. p. palpalis*	Zaïre	Autonomous colony in stationary phase
Offeri et al.	IAEA, Vienna, Austria	May–Dec 1972	*G. tachinoides*	Maisons-Alfort, France	Rearing stopped June 1974
Itard	IEMVT, Maisons-Alfort, France	July 1972	*G. p. gambiensis*	Upper Volta	Autonomous colony in stationary phase
Jordan	TRL, Langford, Bristol, U.K.	Sept 1973	*G. m. morsitans*	Tanzania	Colony in the expansion phase
Offeri et al.	IAEA, Vienna, Austria	Oct–Nov 1973	*G. m. morsitans*	Tanzania	Colony in the expansion phase
van der Vloedt et al.	IAEA, Vienna, Austria	June 1974	*G. p. palpalis*	Antwerp (Belgium) and Nigeria	Colony in the expansion phase

two generations. During the same period, attempts by the same investigator to raise *G. morsitans* and *G. pallidipes* were no more successful, although one colony of *G. austeni* was maintained until the fifth generation with a high degree of reproductive activity. Also in Tanzania, Foster (1957) was unable to maintain *G. morsitans* beyond the third generation.

In contrast, MacDonald (1960), working in Nigeria, succeeded in rearing *G. m. submorsitans* over a 15-month period. This production was only terminated by his departure from Kaduna. Between 1960 and 1972, Gruvel (1970a) and Gruvel and Balis (1966) succeeded in maintaining a colony of *G. tachinoides* in Chad. Conditions were difficult, and the low percentages of survival required frequent input of "wild" puparia and adults. This operation was interrupted in 1972, following the storage of pesticides in the insectary's vicinity.

In the second half of 1964, a fully closed colony was set up at the University of Rhodesia, Salisbury, by Dr D.A. Dame, from flies that had emerged from puparia collected in the Zambezi Valley near Kariba. At that time the species was referred to as *G. m. orientalis* Vanderplank, but since the recent revision of the genus it has become known as *G. m. morsitans* Westwood. It has not been possible to establish unequivocally just how many flies were used to establish the colony, but Mr Lloyd Tsoka (who has been in charge of this colony from the outset), has indicated via Prof E. Bursell of the Department of Zoology, University of Rhodesia, Salisbury,[14] that there was much initial difficulty and that "a very large number" of puparia had probably been brought in from the field before self-sustaining status was achieved after 3–4 months. With the departure of Dr Dame for the USA in 1969, the colony was taken over by Prof Bursell. Ever since the colony's establishment it has been nourished on guinea pigs according to the methods described in Dame and Ford (1966). It has been entirely self-maintaining and has at no time required or received replenishment from the field. Routinely about 40 cages are maintained in the colony, maintenance being discontinued after the 14th week of adult life.

Full records of the colony's performance have been maintained since 1969. During this time, recorded abortions have been steady at about 2% and puparial mortality at about 4%. The number of puparia produced per cage of (initially) 25 females has shown a steady increase from 7 to 14. With mortality at the end of 14 weeks averaging about 60%, a production of 14 puparia/cage/week is close to the theoretical maximum at a temperature of about 25 °C.

Samples of puparia are taken regularly on Monday mornings, larvae having been deposited over the weekend. They would be expected to have lost about 5% of their initial weight by this time. Mean recorded weights have been 27–30 mg since the introduction of Sunday feeding in 1970. In 1973 a program of selection for size was initiated and puparia (male and female) weighing less than 29 mg ceased to be used for breeding. Over a period of about 6 months average puparial weight rose to 32 mg, and weights have remained steady at about this level since late 1974.

These progressive changes in performance, against a background of constant conditions of maintenance, suggest that a process of selection has been at work. This has been confirmed by an experiment undertaken to compare the performance of normal laboratory females with that of females reared in the laboratory from matings with males brought from the field (as puparia). The "hybrid" flies showed significantly higher levels of mortality (50% mortality in 11 as compared with 14 weeks) and regularly lower puparial weights (28.5 mg as compared with 31.0 mg).

East African Trypanosomiasis Research Organization (EATRO) entomologists

[14] Who kindly supplied the contribution on the Salisbury colony (ed).

achieved only indifferent results with *G. morsitans* imported from Tanzania (Moloo and Kutuza 1969), but nevertheless succeeded in raising *G. pallidipes* for several years (Rogers and Kenyanjui 1972). For some years, Challier (1970) and Laveissière (1973) have maintained a low level of production of *G. p. gambiensis* at Bobo-Dioulasso, Upper Volta, for experimental purposes.

Finally, important levels of production are now being achieved in: The International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya — *G. m. morsitans* and *G. austeni*; The National Council for Scientific Research (NCSR), Lusaka, Zambia — *G. m. morsitans*; The Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna, Nigeria — *G. m. submorsitans*; The Tsetse Research Project, Tanga, Tanzania *G. m. morsitans* (Williamson 1974); and the Institut d'Elevage et de Médecine Vétérinaire des Pays Tropicaux (IEMVT), Bobo-Dioulasso, Upper Volta—*G. p. gambiensis* (Clair 1975).

European Colonies

All the tsetse species reared in European laboratories belong to the subgenera *Glossina* (= *morsitans* group) and *Nemorhina* (= *palpalis* group) (Table 15). There is at present no laboratory production of species belonging to the subgenus *Austenina* (= *fusca* group), although attempts at rearing species of this group were made between 1962 and 1970 in Africa and Europe.

During 1962, in Nigeria, Jordan (1962a, b), using simple installations, succeeded in rearing *G. tabaniformis, G. medicorum*, and *G. fusca*. After those limited attempts he concluded that flies of the *fusca* group could be reared as easily as the species of other groups. However, it was only with much difficulty that Finelle and Lacotte (1966) succeeded in maintaining a culture of *G. f. congolensis* in the Central African Republic (1965–66). The rearing of the latter species was also attempted in the IEMVT laboratory of Maisons-Alfort (IEMVT 1969, 1970). The first experiments carried out in 1968–69 with puparia from the Central African Republic did not succeed despite the fact that those adults hatched had a sufficient life span. This was due to the very low reproductive rate of the females, and considerable puparial mortality, shortly before hatching. A second attempt carried out in 1970 with *G. f. congolensis* collected in Uganda was also unsuccessful. The females hatched in the laboratory failed to reproduce satisfactorily.

Switzerland

Rearing techniques for tsetse flies progressed considerably when Geigy (1948) succeeded in maintaining a breeding colony of *G. palpalis* (*G. f. quanzensis*) for more than 5 years at the Swiss Tropical Institute, Basel. Adult flies (77 males and 211 females) caught in the surroundings of Brazzaville (Congo) in October 1945 were engorged and then transported by airplane in a freezer maintained between 8 and 15 °C. Upon arrival at Basel, 3 days later, there were only 22 males and 98 females alive. These flies were used to initiate the colony. The adult flies were kept in cages measuring 14 × 8 × 4.5 cm (Roubaud modified system) enclosed with a 3-mm mesh cotton material. Each cage contained 8 females and 6 males. The larvae were deposited on the bottom of the cages. After passing through the netting, the larvae formed puparia and were then collected. Feedings, which lasted 45 minutes, were made every day, except Sunday, from four guinea pigs maintained in an ingenious device that allowed the cages to be applied to the flanks of the guinea pigs (2 cages each). Until 1947, the flies were reared at 26 °C, and the humidity was maintained at about 80% by means of wet rags. This temporary installation afforded no temperature control; therefore, in August 1947, the colony was moved to an air-conditioned room that ensured a continuous circula-

Immobilized guinea pigs being exposed to caged G. m. submorsitans in laboratory of F. de Azevedo, Lisbon, Portugal, 6 Feb 1965 (M. Laird).

tion of the air as well as a temperature of 26 °C and a humidity of 85%. The room was automatically lit by fluorescent lights from 6:00 until 18:30.

The tsetse population increased more or less regularly until May 1947, when it reached a little more than 1400 flies. From June to December 1947, the population decreased to 250 individuals, and then rose again to 1400 (800 females and 600 males) in September 1948. Although Geigy did not publish any further observations, Nash (1963) mentioned that this rearing had then survived for about 5.5 years.

Geigy also tried to rear *Glossina* from puparia imported from Kinshasa, Zaïre (4 shipments) and Entebbe, Uganda (1 shipment). However, these populations could not be maintained for more than 19 months for the largest shipment (180 puparia), and only 4–6 months for the other shipments.

Portugal

The second successful attempt to establish closed colonies took place at Lisbon's Institute of Tropical Medicine (now renamed the National School of Public Health and Tropical Medicine). Azevedo and Pinhão (1964a,b) used 238 *G. m. morsitans* puparia (imported from Mozambique in September and October 1959), from which 21 males and 22 females hatched to become the colony's parent generation. The output remained fairly stable at a low level for 4 years. It was only after November 1963 that the population began to increase at an appreciable and uniform rate, the number of adult tsetse rising in 1 year from 21 to 1000. Azevedo and Pinhão (1964a,b) attributed their success to a new rearing technique, with particular respect to keeping the puparia in wet sand.

In April 1965, the rearing of *G. m. submorsitans* was undertaken with 83 puparia collected in Nigeria (Azevedo and Pinhão 1967); and in July 1966, a colony of *G. m. morsitans* was established from 1800 puparia collected in Rhodesia (Azevedo et al. 1968). From the beginning, this *G. m. morsitans* colony developed much better than the colony of *G. m. morsitans* from Mozambique (Pinhão et al. 1970). On the other hand, the Rhodesian colony of *G. m. morsitans* (p. 127) progressed at a much slower rate (Dame et al. 1970).

These three colonies prospered until 1971, when they were destroyed by an insecticide spraying carried out in the vicinity of the laboratory (Azevedo 1971). In 1969, efforts were initiated to colonize *G. pallidipes* (starting with 400 puparia from Kariba, Rhodesia); this attempt failed, whether from insecticide contamination, physical shortcomings, or inadequate insemination is unclear (Azevedo and Guerreiro 1971). Since February 1971, a small colony of *G. m. submorsitans*, originating from Nigeria and comprising 600 females and 200 males, has been maintained at Lisbon's Institute of Hygiene and Tropical Medicine (Pinhão, personal communication 1975).

The United Kingdom

A closed colony was established at Langford, near Bristol, England, at the Tsetse Research Laboratory. Using special packing techniques (Kernaghan and Nash 1964), the Langford scientists imported, between June 1963 and April 1966, 49 000 *G. austeni* puparia collected in Zanzibar (now in Tanzania) (Nash and Jordan 1970). From December 1965, the colony grew spectacularly. Since April 1966, it has been entirely closed and has shown a large surplus.

In February and March 1967, a colony of *G. m. morsitans* was established from tsetse (initially 94 females) that emerged from puparia collected in Rhodesia. There was further importation of puparia, and in September 1969, the population included 3200 females (Jordan et al. 1970).

In September 1973, the production of *G. m. morsitans* was initiated with flies (including 146 females) emerging from puparia collected in Tanzania (Jordan 1974a, b).

France

In August 1964, a colony was started at the IEMVT entomology laboratory at Maisons-Alfort, from the flies yielded by 326 *G. m. centralis* puparia collected in July 1964 in Tanzania (Itard and Maillot 1966). This colony, initially having 68 females, progressed very slowly. There were still no more than 160 females in December 1965. After being used for various experiments, this colony was abandoned in November 1967.

In 1965, the IEMVT investigators received from Rhodesia 1110 *G. m. morsitans* from which 446 adults (220 males and 226 females) hatched. The colony remained stable throughout 1966 (averaging 360 females). It was only during 1967, after the introduction of rabbits as the source of blood, that the numbers increased sharply, from 455 females in January to 2710 females in December (Itard 1971a).

The first attempts at rearing *G. tachinoides* were made in April 1965 from 124 puparia collected in Chad. This isolate survived with difficulty. Although a new shipment of 147 puparia was received in February 1966, the colony still had only 28 females in October 1966. In November and December 1966, a further shipment of 3162 *G. tachinoides* puparia from Chad and the use of rabbits for feeding purposes, gave the colony new impetus. Despite an accidental insecticide exposure in June 1967, the numbers reached 2105 females in December of the same year (Itard et. al. 1968).

In October 1966 and January 1967, the Langford laboratory shipped 249 puparia of *G. austeni* to Maisons-Alfort. From these, a colony was initiated. Starting with 19 females in January 1967, it remained

stable at a low level for nearly 8 months. The numbers started to increase in September 1967, when 60 females were present. There were more than 1000 females in May 1968, and 2000 in November 1968 (Itard 1971a).

The rearing of *G. f. fuscipes* began in June 1968, using the flies emerging from 13 puparia collected in the Central African Republic. Two other lots of 39 and 43 puparia of the same origin were received in November 1968 and January 1969. These three shipments originated the *G. f. fuscipes* strain of Maisons-Alfort (Itard 1971a). The colony progressed until February 1970, when the stock grew to 1125 females. Later, there was a decline in numbers, and by July 1970 only 556 females remained. The population then increased quite rapidly, reaching 2132 females in March 1971, and 4826 by September of the same year. The decline in numbers experienced during early 1970 may have been due to inbreeding, because an increasing number of individuals exhibited lethal genes in the homozygous state (Itard 1971a).

The rearing of *G. p. gambiensis* began in July 1972 from two lots of wild females and puparia collected in Upper Volta. In August 1972, there were 54 females in the colony. By October 1972, the total had fallen to 47. After this, the colony developed at a uniform rate. It contained more than 1000 females by December 1973 (Itard 1975a), and reached 4700 in July 1974. Since August 1974, the number has been kept constant at about 4800 by withdrawing the surplus offspring — about 3000 puparia and 180 females monthly (Itard 1976).

Belgium

Rearing attempts started as early as 1966 at the Laboratoire d'Ecologie du Rijksuniversitair Centrum of Antwerp. Initially, these efforts depended upon the availability of *G. morsitans* puparia from Portuguese sources in Africa, and from Rhodesia. This colony never exceeded 60 females. It suffered (June–August 1967) a slow intoxication by insecticide that almost completely destroyed it (Van der Vloedt et al. 1968). *G. m. morsitans* puparia collected in Rhodesia in June and October 1967, and March, May, and October 1968 (Van der Vloedt 1970; Van der Vloedt et al. 1968) enabled the reinstatement of this colony. It was, however, discontinued after 1970.

In February 1968, the rearing of *G. p. palpalis* was undertaken from five females imported from Zaïre. This colony developed uneventfully. By September 1970, it included 2750 females (Van der Vloedt 1971).

During 1971, researchers at Antwerp, noticing a decrease in production, a noticeable increase in both the death rate (over 5% per day) and the proportion of sterile females, established a second independent colony from female *G. p. palpalis* caught in lower Zaïre from December 1971 to January 1972. The rate of increase of this colony was very satisfactory and the population was maintained, during 1974, at an average of 2500 females (Van der Vloedt, personal communication 1975).

In April 1968, from 53 female *G. f. quanzensis* captured near Brazzaville (Congo), a third colony was established, which also developed uneventfully. The population reached close to 250 females in February 1969 (Evens and Van der Vloedt 1970b), and produced 1250 females per day at the fifteenth generation (Van der Vloedt, personal communication 1975).

Austria

The International Atomic Energy Agency (IAEA) initiated the rearing of *Glossina* with a view to developing mass-rearing techniques, studying artificial feeding through membranes, and obtaining flies that could be used in control programs involving the release of sterile males (Wetzel et al. 1975).

Production was undertaken in late 1968 and early 1969 with *G. austeni* puparia

from the Langford laboratory (United Kingdom), and continued until mid-1970.

Early in 1969, a colony of *G. m. morsitans* was established from puparia obtained from the Lisbon rearing, and directly from Rhodesia. This colony developed with excellent reproduction and longevity rates. However, numbers have always remained quite moderate — less than 1000 females in March 1974, for the strain fed on live animals, and less than 700 for the strain fed through a membrane (Wetzel 1974).

Several lots of *G. tachinoides* puparia shipped from IEMVT's Maisons-Alfort laboratory (May–October 1972), enabled the establishment of a colony initially including 600 females. This population grew rapidly, but was reduced to about 1000 females at the end of April 1973, and held at this level until December 1973. It was discontinued in June 1974 (Offeri and Dorner 1974).

Finally, a culture of *G. m. morsitans* was started at the end of 1973 from puparia collected in Tanzania. The colony, which initially had 54 parous females, included 137 females in March 1974 (Offeri and Dorner 1974).

Other Colonies

The availability of flies, particularly from Langford, has led to an important stimulation of physiological studies. In Canada, for example, a small colony of *G. austeni* was maintained first at the Institute of Parasitology of McGill University in Montreal and later transferred to York University in Downsview, Ontario. *G. m. morsitans* is also reared at the University of Toronto. These colonies are intended to produce limited numbers of flies for physiological studies, and are not closed, being augmented at frequent intervals by puparia from the parent colony in Langford. The flies are fed on rabbits on alternate days (Tobe and Davey 1971).

Since this section was prepared, Dr G. Poinar, Jr has reported parasitological studies undertaken with *G. m. morsitans* reared at the Department of Entomology, University of Amsterdam.

Basic Conditions for Tsetse Colonizations

The factors governing tsetse colonization are closely related to the biology and ecology of these insects. Success depends on the observance of rules already published by various authors (Evens 1964; Nash 1963; Lumsden and Saunders 1966; Itard 1971b,d).

Since both sexes of tsetse are strictly haematophagous, proper food is essential. Following experiments with various species of mammals, research workers generally feed their colonies on guinea pigs, rabbits, or goats. Experiments on artificial feeding through a membrane with haemolyzed or defibrinated blood are also being carried out in various laboratories.

The number of meals varies according to the temperature of the environment, and the physiological state of the flies. In practice, the insects being reared must be given the opportunity to feed every day. With well-established colonies, however, it is possible to omit feeding 1 day each week. When the flies are fed on live animals, care must be taken to avoid cutaneous reactions in the host animal subjected to too-frequent bites (Nash 1970a; Nash et al. 1965). This is achieved by ensuring that each animal is rested for at least 3 days between utilization periods.

Female tsetse are larviparous. The first larva is deposited at about 18–20 days, with subsequent ones following it at regular intervals, averaging 10 days. Under optimum conditions the mean life span in the laboratory is about 100–150 days; therefore, a female's average lifetime production of larvae is 10–15. The growth rate of a population is therefore very slow. Consequently, it is important to provide the females with optimum living condi-

tions and as high an insemination rate as possible.

The most favourable conditions are achieved, depending on the species, with a temperature of 24–26 °C and a relative humidity of 60–85%. Temperatures above 27–28 °C lead to a high mortality among both puparia and adults, and the cessation of larviposition by surviving fertilized females. Puparia are generally maintained at slightly lower temperatures than adults (23.5–25.5 °C) and at a somewhat higher relative humidity (80–90%). Under these conditions, the average duration of the puparial stage is (depending on sex and species) 27–37 days; the hatching rate exceeds 92%.

Females mate when 3 days old with males at least 7 days old. In general, groups of 10–25 females are caged with an equal, or slightly superior, number of males for a period of 24–72 h, after which the sexes are separated. With this technique, insemination rates are always above 80%, and usually between 86 and 96%.

Different investigators have favoured different light regimes. Generally, though, a rhythm is adopted of more or less intense artificial lighting during the day with the flies remaining in the dark from 18:00 until 6:00.

One of the major causes of failure in tsetse rearing is accidental contamination by insecticides (Azevedo 1972; Nash et al. 1971). It is often very difficult to track down the source of such contamination, the effects of which may be insidious and not detected for many days after the intoxication has started. The insecticide may be introduced into the rearing chamber by blood-donor animals (Azevedo and Pinhão 1967; Nash et al. 1971; Van der Vloedt et al. 1968). There may also be contamination via attendants' or visitors' clothing, or maintenance material and products. A close watch is therefore necessary to ensure that the use of all insecticides is banned in the vicinity of the insectary. Electrocution traps with a UV attractant (Nash and Jordan 1971) may be used to destroy winged insects seasonally invading the rooms where the host animals are kept, and where tsetse flies are reared.[15] Moreover, the thorough washing of newly introduced feeder animals is recommended.

Paint fumes, formaldehyde contained in the glue of laminated wood and some varnishes, and tobacco smoke are all dangerous to tsetse. Chlorine is also harmful, and the material used in the insectary, particularly the cage netting, must be washed with ordinary white soap and carefully rinsed with demineralized water.

Another cause of intoxication, resulting in a lower fecundity rate in females, originates from certain medicaments administered to the feeder animals or contained in their food (Jordan and Trewern 1973; Turner and Marashi 1973).

Comparison of Different Techniques

Feeding

In all laboratories the flies are fed on live animals 6 days a week, the main differences being in the choice of feeder animals. In addition, at Langford and Seibersdorf, artificial feeding techniques through membranes are being developed for the maintenance of some species in closed colonies.

On Live Animals

Langford Laboratory

The Langford colonies are fed in the proportion of 11% for *G. morsitans* and 21% for *G. austeni* on lop-eared rabbits weighing, on the average, 3–4 kg. The rest are fed on the flanks of large (European) goats. Other animals (sheep, pigs, calves)

[15] Adequate screening of the facility to ensure no escapes of adult tsetse should preclude such seasonal invasions too (ed).

have also been used on an experimental basis.

Rabbits — The Langford group was able to obtain (for the first time with tsetse) a virtually maximum rate of reproduction, by feeding their *G. austeni* colonies at the ears of rabbits (Nash et al. 1966; Jordan et al. 1967).

Initially, a cage containing 10 flies was placed on each ear of a rabbit for 15 minutes. Later, the feeding time was reduced to 10 minutes and the number of flies per cage was increased to 15.

Three groups of rabbits are used per week, each group providing food for the flies one day out of three (or four when the third day is not a working day). The number of flies placed on each rabbit never exceeds 160 per day. About half of these flies actually feed (Jordan et al. 1967). The possibility of increasing the number of flies without harming the rabbit, or decreasing the longevity and fecundity of the tsetse flies, is being investigated. In the case of *G. morsitans*, 270–300 flies are an acceptable number; for *G. austeni*, 400–450 is the maximum tolerable limit (Nash et al. 1971).

Using a daily maximum of 160 flies per rabbit, the eventual puparial yield was 8–9 for each female *G. austeni*, the individual puparia averaging 23–24 mg. This is equivalent to the average weight of newly formed puparia collected in the field. Feeding on rabbits gives equally good results with *G. morsitans*, and this technique has also been used with success at Maisons-Alfort with other species of *Glossina* (Itard 1975a; Itard and Maillot 1970).

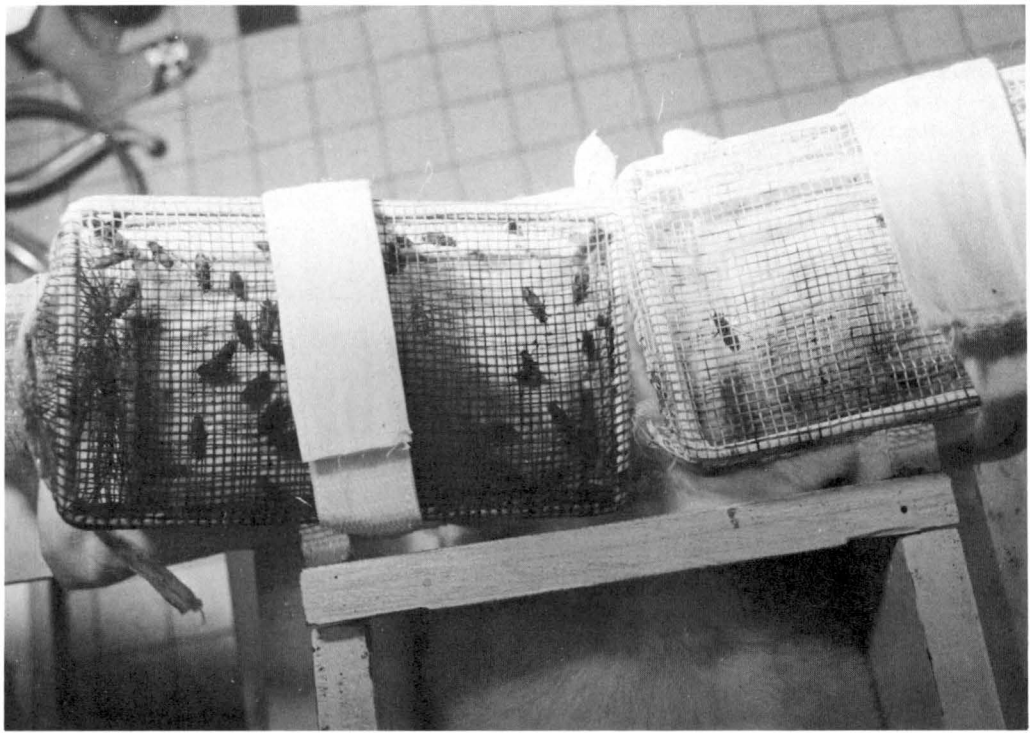
Goats — These animals give very satisfactory results at Langford. Six cages, 25.4 × 12.7 × 5.0 cm, covered with black terylene netting and containing 25 flies per cage, are placed on the flanks of the goats (3 cages on each side) for 15 minutes. The flanks of the goats are sheared every 15 days, but not shaved. All animals are washed with soap and warm water every other month. Moreover, each goat is tested at about 4-month intervals to determine its capacity as a feeder host. The proportion of fasting 2-day-old males that feed on the animal provides a "host-capacity index," which must not be below 80%. Incapacity on the host's part may be due to sensitivity to tsetse saliva, to thickness or hardening of the skin, or to a poor general condition due to old age or parturition (Nash et al. 1968).

A 73-kg adult goat can thus feed 800 flies every 3 days. The cages are secured on the sides of the goat by means of rubber bands in such a way as to ensure a close contact with the skin surface. Using this method, the Langford group regularly achieves a production of 7–8 puparia weighing 23–24 mg from each female *G. austeni*, and 5–6 puparia weighing about 30 mg for *G. morsitans*. These weights are comparable to those of naturally produced puparia.

Calves, sheep and *pigs* — Research carried out at Langford on the use of large animals as feeder hosts has shown that, whereas pigs and sheep are difficult to handle, feeding on calves gives satisfactory results. However, wastes from these animals, and their handling, create problems under laboratory conditions in Europe. In Africa, where climatic conditions allow the feeding of flies in the open air, the use of calves, or even adult cattle, may present less difficulty (Jordan et al. 1966).

Maisons-Alfort Laboratory

After the initial use of guinea pigs as feeder animals (Itard and Maillot 1966), all colonies were fed on the ears of rabbits (Itard 1971b; Itard et al. 1968), as at Langford, from January 1967 onwards. A feeding opportunity was presented daily, except on Sundays. At present, groups of nine adult rabbits of the "bouscat" race, weighing 5–7 kg, are each used 1 day a week, and allowed to rest during the following 6 days. The cages (14 × 8.5 × 5 cm), containing 30–35 flies, are kept on the rabbits' ears for only 4–5 minutes. This permits the feeding of all colonies (22 000 flies approximately) in one morning. As



Tsetse being fed on ears of rabbits, IEMVT, Maisons-Alfort, 27 June 1973 (J. Itard).

each fly averages one meal every 2 or 3 days, a single rabbit therefore feeds 1000–1200 flies one morning a week.

Rabbits do not seem to suffer from this schedule. They do not lose weight, longevity is adequate, and the only causes of mortality observed are infections by *Pasteurella*, which is endemic in the hutches. However, longevity and reproduction rates of puparia of *G. austeni* and *G. m. morsitans* are relatively lower than those achieved at Langford with the same species.

Seibersdorf Laboratory

The flies are fed daily (except on Sunday) on rabbit ears, for 5 minutes at a time, as at Maisons-Alfort. However, each rabbit is used every 3 days. On the average, 300 flies are fed on one rabbit, but this figure is sometimes much higher (Mews et al. 1971).

Antwerp Laboratory

Feeding is carried out daily, except Sunday, exclusively on guinea pigs. These, their hair clipped with scissors, are immobilized on a holding board equipped with a drawer for collecting faeces and urine. The cages, each containing 1–10 flies, are placed between two guinea pigs for a period of 0.5 h (Evens and Van der Vloedt 1970a).

Artificial Feeding

Mainly at Langford and Seibersdorf, attempts have been made to feed tsetse through an artificial membrane on blood, which has been rendered uncoagulable. The eventual objective of these investigations is the replacement of blood by a chemically defined artificial medium. Unsuccessful experiments were undertaken at Langford (Langley 1972) to maintain

closed colonies of *G. morsitans* on haemolyzed ox blood, and at Seibersdorf (Mews et al. 1976a,b) using fresh defibrinated bovine blood. However, small self-maintaining colonies of this species were established in both laboratories by feeding flies 5 days a week through a membrane, and 1 day on live rabbits (Langley and Pimley 1976; Mews 1975; Mews et al. 1976a). Production rates of female *G. morsitans* fed in this manner were good (6–7 puparia per female), but the weight of the puparia, 26–27 mg, was about 10% lower than that of those produced by females fed on live animals.

At Maisons-Alfort, experiments with artificial feeding on citrated-glucosified sheep blood through a silicone synthetic membrane were also undertaken with females of *G. austeni* and *G. morsitans*. Although longevity and larviposition of females fed artificially are quite good, the puparial weights are also nearly 10% lower than those of puparia produced by females fed on rabbits (Pagot et al. 1973).

Recently, the problem of low weights of puparia produced by female tsetse fed artificially seems to have been overcome at Langford by feeding the flies on fresh defibrinated pig blood, without the need of supplementing this diet by a 1-day-a-week feeding on a live animal (Mews et al. 1976a). The fly cages consist of a plastic cylinder 4.5 cm high and 12.5 cm in diameter, closed by stretched black terylene netting. The blood, placed on grooved glass plates kept at a temperature of 40 °C, is covered by a well stretched parafilm membrane on which rests a second agar membrane, 1.5 mm thick. The cages, each containing 15 females, are left in contact with the agar membrane for 15 minutes. By using only this feeding method a closed colony having about 5000 females was established. Each female produced an average of 5–6 puparia weighing 30 mg.

Perfect sterile conditions are essential when artificial feeding techniques are used. If these aseptic conditions are not achieved, heavy mortality occurs among the flies (p. 145). Their abdomens become black, and the intestinal track exhibits bacteria of the genera *Pseudomonas, Aeromonas, Flavobacterium, Micrococcus*, etc., which cause death (Bauer 1974).

Maintenance of Colonies

Climatic Conditions

Langford Laboratory

Adults are kept at a temperature of 25 °C; humidity rates are 70–80% for *G. austeni* and 60–70% for *G. morsitans*. Puparia are kept at 23.5 °C. A lighting rhythm of 12 h (5–27 lux) followed by 12 h of darkness is maintained in the insectary.

Maisons-Alfort Laboratory

Temperature throughout the insectary is maintained at 25 °C ± 1 C°. Humidity rates vary from 60 to 80%, depending on the species; puparia are kept in a small room at 80–85% relative humidity.

The air, constantly renewed, is aspirated from the outside, then evacuated by an automatic extractor through metal sheaths equipped with adjustable flaps. Lighting is provided by neon lamps that are manually controlled, with the exception of one programmed by an electric clock that cuts the circuit at 18:00 and reestablishes it at 6:00.

Seibersdorf Laboratory

The rearing rooms are maintained at a constant temperature of 25 °C ± 0.5 C° and a relative humidity of 60 ± 10%. The air is renewed about 2.5 times an hour, and is circulated through the insectary by means of small ventilators.

Antwerp Laboratory

Forced air circulated through vapour from boiling water gives a relative humidity of 80–85%, which can be raised to 95%. To maintain the temperature in the vicinity of 25–26 °C, air cooled by freon pipes is introduced from the ceiling.

Collection of Newly Hatched Flies

Langford Laboratory

Puparia are kept on dry sand in aluminium tanks (40 × 8.2 × 2.5 cm) placed in a large hatching cage covered with a removeable stainless steel frame (40 × 35 × 10 cm) covered with black terylene netting. Hatched flies are collected every day, immobilized by cooling to 2–4 °C, sexed, and placed in cages. Hatching rates vary from 96 to 98%.

Maisons-Alfort Laboratory

Puparia, collected each morning, are immediately placed in 8 cm sterile glass tubes 3.6 cm in diameter. Thirty to thirty-five puparia are introduced into each tube, and no sand is added. The tubes are covered with a square of tergal netting kept in place by a rubber band and stored in a small room until hatching takes place. Hatched flies are released in a large cage, collected by means of a test tube, sexed, and placed in cages according to sex and species. Hatching rates exceed 92% in all cases (overall mean = 94%).

Seibersdorf Laboratory

A technique similar to the one at Langford is used. Hatching rates are of the order of 95–96%.

Antwerp Laboratory

The technique generally resembles that used at Maisons-Alfort, except that the bottoms of the glass jars are covered with sterile sand in which puparia are buried. Hatching rates vary, according to species, from 79.6% (*G. f. quanzensis*) to 94% (*G. p. palpalis*).

Mating

Very similar techniques are used in the four laboratories. Groups of 1–25 females, 3-days-old (3–6 days at Antwerp), are placed with an equal or slightly larger number of males, at least 7 days old (5 days at Antwerp); sexes are separated after 24–72 h (1 week at Antwerp).

The separation of sexes is carried out in a cold (2–4 °C) atmosphere at Langford, and in a CO_2 atmosphere at Seibersdorf.

However, it was found at Langford that, with this method, insemination rates were low for *G. austeni*. They were higher when one male was introduced into a tube containing only one female. At Maisons-Alfort, on the other hand, *G. austeni* (like the other species reared) is mated in groups (10–15 females with as many males).

Maintenance of Reproductive Females

Females are kept in cages made of a stainless metal frame covered with netting (black terylene at Langford, white tergal at Maisons-Alfort) with meshes of a size allowing the passage of deposited larvae, but not of adults. The dimensions of these cages and the number of females per cage vary between laboratories.

At Langford two types of cages are used: large ones, 25.4 × 12.7 × 5.1 cm, containing 25 females fed on goats, and small ones, 15 × 8.5 × 5 cm, containing 15 females fed on rabbits. At Maisons-Alfort only small cages (14 × 8.5 × 5 cm) are used, each containing 30–35 females. At Antwerp the cages (15 × 10 × 17 cm), covered with cotton netting, contain 10 females. At Seibersdorf, cages are made of polyvinyl chloride tube sections of varying dimensions. The section surfaces are covered with black terylene netting glued to the periphery of the tube (Mews et al. 1971).

At Langford and Maisons-Alfort the cages are aligned, in groups of 10, in metal supports (Itard and Gruvel 1969; Nash et al. 1971), under each of which is a drawer containing sand or a sheet of tissue paper. The larvae pass through the mesh of the cages and fall into the drawer where they metamorphose into puparia. All that has to be done is to remove the drawer each morning and collect the puparia. The use

Table 16. Coefficient of natural growth (r_m) for species reared in Europe.

Laboratories	Species	Feeder animals	r_m Values	Observations
Tsetse Research Laboratory, Langford, U.K.	*G. austeni*	Rabbit	0.0166	from Jordan and Curtis 1968
	G. austeni	Rabbit	0.0173	from Curtis and Jordan 1970
	G. austeni	Goat	0.0157	from Curtis and Jordan 1970
	G. m. morsitans	Rabbit	0.0166	from Jordan and Curtis 1972
	G. m. morsitans	Goat	0.0145	from Jordan and Curtis 1972
IEMVT, Maisons-Alfort, France	*G. tachinoides*	Rabbit	0.0125	r_m values obtained from equation for actual growth curves of populations
	G. f. fuscipes	Rabbit	0.0082	
	G. p. gambiensis	Rabbit	0.0083	
Laboratorium voor Oekologie, Antwerp, Belgium	*G. p. palpalis*	Guinea pig	0.0092	from Van der Vloedt 1975
	G. f. quanzensis	Guinea pig	0.0077	from Van der Vloedt 1975

of this apparatus permits a large number of females to be stocked in a limited space, resulting in a significant saving of time in puparial harvesting. At Seibersdorf and Antwerp the cages are placed in plastic tubs, into which the larvae fall.

Productivity of Laboratory-Reared Tsetse

The productivity of tsetse fed on animals was examined by Jordan and Curtis (1968, 1972) and Curtis and Jordan (1970). Colonies are said to be in the "expansion phase" when all the offspring produced are reintroduced into the culture. The growth of such a colony is then exponential. Its growth rate is represented by the formula:

$$N_t = N_o e^{r_m t}$$

in which: t = time interval in days; N_o = number of females at time 0; N_t = number of females at time t; and r_m = coefficient of natural growth. This value, characteristic of the species considered, is also a function of the conditions under which the population is raised.

Some values of r_m for the species reared in Europe are given in Table 16. Important differences from one species to another, and also according to the rearing methods used, can be seen. The species in which the r_m values are the highest are those of the *morsitans* group reared at Langford. It is well known that the species belonging to this group can be reared much more easily than those of the *palpalis* group, the reproduction rates of which are lower. Moreover, the r_m values calculated by the Langford investigations are obtained from life tables for females, which give probabilities of survival at different ages, and from fecundity tables in relation to the age of females. Maximum growth rates of colonies in the expansion phase are thus obtained.

In contrast, the r_m values given for the species of the *palpalis* group reared at Maisons-Alfort were derived directly from the growth-rate equation using the actual numbers of females at the time the colonies were in the expansion phase. These values, therefore, do not represent the maximum growth rates of the species. Under these conditions, however, the values arrived at for *G. tachinoides* are significantly higher than those for other species of the *palpalis* group. This species has in fact a better reproductive rate, close to that of the species of the *morsitans* group.

The r_m values computed at Antwerp for *G. p. palpalis* and *G. f. quanzensis*, were calculated from actual data collected from individual rearings of these species (Evens, personal communication).

From the r_m values it is possible to derive the time required for the population to double (a constant factor in the case of exponential growth) and also to make pre-

Table 17. Alternative measures of the maximum rate of increase of expanding colonies of *G. morsitans* (from Jordan and Curtis 1972).

Method of raising *G. morsitans*	r_m	Doubling time	Increase in 1 year
Goat-fed	0.0145	47.8 days	199-fold
Rabbit-fed	0.0166	41.7 days	428-fold

dictions from one to several years in advance. Table 17 shows the various parameters established by Jordan and Curtis (1972) with their colony of *G. m. morsitans* in the expansion phase. Various factors significantly affect the course of development of a population. The main ones were analyzed by Pinhão (1972). Generally, the daily mortality rate must not exceed 2% of the colony (Jordan 1975).

From the practical point of view, it is interesting to know the productivity of colonies in the "stationary phase." A colony is in the "stationary phase" when, having attained a given size, the numbers are maintained at a constant level by removing the excess progeny, which thus become available for various research projects. The yield of such a colony can be measured simply in terms of excess progeny that can be removed from the colony without reducing the number of females.

In 1973, the Langford rearing included an average complement of 7700 females (4500 females of *G. morsitans* and 3200 females of *G. austeni*) fed on animals: 91% on goats, the balance on rabbits. These females produced, during the same year, 220 800 puparia, of which 123 800 were used for research and not returned to the colony (Table 18).

At Maisons-Alfort, during the same year, the colony included a total of 13 670 females, excluding the females of *G. p. gambiensis*, the rearing of which was then in the expansion phase. A total of 272 000 puparia (excluding those of *G. p. gambiensis*) were produced. The excess production was 110 500 individuals (Table 18). Thus, these two colonies gave a surplus representing about half of their annual production (56% at Langford; 41% at Maisons-Alfort).

Jordan and Curtis (1972) estimated the numbers that can be removed weekly from a colony of 100 adults of *G. morsitans* in the stationary phase (Table 19). In column 1 is shown the weekly production of viable puparia of each sex from such a colony (number of puparia multiplied by the observed hatching rate). Columns 2 and 3 show the numbers of adults that must be reintroduced into the colony to replace the dead ones. Since one

Table 18. Productivity of glossinid rearings at Langford and Maisons-Alfort in 1973.

Laboratories	Species reared	Average number of females	Annual puparial production	Annual surplus of production (puparia + adults)
Tsetse Research Laboratory, Langford, Bristol, U.K.	*G. austeni*	3200	92700	57400
	G. m. morsitans	4500	128100	66400
		7400	220800	123800
Institut d'Elevage et de Médecine Vétérinaire des Pays Tropicaux, Maisons-Alfort, France	*G. austeni*	3300	75850	39050
	G. m. morsitans	2670	56780	25250
	G. tachinoides	4360	85590	19570
	G. f. fuscipes	3340	53820	26600
		13670	272040	110470

Table 19. Estimates of weekly inputs for stationary colonies of 1000 adult *G. morsitans* (from Jordan and Curtis 1972).

Method of raising *G. morsitans*	Total production of viable puparia of each sex (1)	Input of viable puparia required to maintain colony		Disposable output in the form of	
		Female (2)	Male (3)	Viable puparia of each sex (4)	Young adult males (5)
Goat-fed	257	76	13	181	244
Rabbit-fed	259	56	9	203	250

male can mate up to six times and since the fecundity of the females successively fertilized by the same male does not decrease (Jordan 1972a), the number of males to be kept is clearly smaller than that of females. The difference between columns 1 and 2 represents the required weekly availability of viable puparia of each sex (column 4). If the individuals are sexed on hatching, the number of males available will in fact prove to be larger. This maximum, calculated by the difference between columns 1 and 3, is shown in column 5.

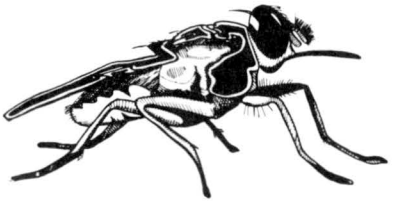
The potential sex of puparia cannot be determined at present. In the case of a colony not yet firmly established, it is therefore necessary to await the hatching of adults before making decisions concerning utilization of the excess production. However, since in a batch of puparia of the same age, the females hatch 1 or 2 days before the males, the remaining puparia, mainly destined to yield males (Curtis and Langley 1972), may be removed from the colony. This will ensure a surplus of males close to the figures shown in column 5, while preserving the advantages inherent to the handling of puparia, which are obviously easier to manipulate than the adults.

Only a decade ago, the colonization of tsetse flies was held to be formidably difficult. However, it is now successfully realized in several laboratories. Techniques have been developed to permit the maintenance of colonies comprising several thousand individuals. The growth rate of these populations usually exceeds that of natural tsetse populations, during the most favourable periods of the year.

The rearing of tsetse flies has become an exact science, not only through the precision of techniques used, but also because mathematical methodology enables assessment of the performance of the colonies and the prediction of the number of individuals that can be removed. These represent approximately one half of the annual production of puparia or, on a weekly basis, between 18 and 25% of the total number of adult flies, depending on the mode of feeding and the stage at which the individuals are withdrawn from the colonies. In practice, however, it is wise to have a margin of security, and to keep a larger number of puparia than that determined mathematically. This precaution compensates for the high mortalities that may occur from time to time, even in the best of rearings.

Costs of rearing tsetse flies will always exceed those of oviparous insects, even if methods of artificial feeding are generally adopted. Because of *Glossina*'s larviparity, productivity in rearing species of this genus is unlikely to surpass the maximum values calculated by the Langford group for *G. austeni* and *G. morsitans*.

The last 10 years of progress is nevertheless important, and the existing productivity is more than adequate to fill research needs. It is desirable that African colonies at least as productive as the European ones be established in the near future, in order that the needs of genetic control field efforts and biological control research are met. — J. Itard and A.M. Jordan.



Mass Production Using *In Vitro* Feeding

The demand for laboratory-reared tsetse flies is increasing rapidly. Only when sufficient biological material is readily and constantly available can efficient experiments on disease transmission, physiology, attractants, field behaviour, etc. be evaluated. In addition, steadily increasing interest in developing nonchemical methods of tsetse control dictates that the rearing of these insects be made more efficient and less expensive.

At present, most tsetse fly colonies reared under laboratory conditions utilize *in vivo* feeding. Goats, rabbits, and guinea pigs are normally used. Procedures used in rearing tsetse flies on animals are described in the previous section. The data presented indicate that after investigators overcome initial difficulties, the rearing of certain species becomes a matter of routine. As with any other insect, certain precautions must be taken for successful laboratory rearing. For several species, the rearing criteria necessary for success are well documented.

The primary difficulty with *in vivo* tsetse fly rearing is that the investigators must maintain not only the insect colony but also a supporting colony of some warm-blooded animal; this requires a division of effort. The space requirements for maintaining the *Glossina* colony are considerably less than those needed with respect to the vertebrates. Also, the maintenance of vertebrate colonies requires considerable veterinary expertise — and entomologists rearing tsetse flies seldom have veterinary training as well.

In Vitro Feeding Techniques

Were simple techniques for *in vitro* feeding available, this would certainly free more technical assistance for actual tsetse-fly rearing. More progress could thus be expected on all fronts in the attack against animal and human trypanosomiases. In particular, an adequate *in vitro* feeding technique would make possible more precise studies of disease transmission, physiology, etc.

Such a technique will not be fully utilized until a stable, readily available food source has been developed. The first steps may involve the availability of whole stored blood or some fraction of it, freeze-dried blood, or an artificial diet made up of relatively commonly available ingredients; to be succeeded eventually by a completely synthetic diet.

Requirements for *In Vitro* Feeding

When colonizing slow-breeding insects like *Glossina* spp., the difference between success and failure is always slight. Therefore, a constant check on colony performance must be kept. This can be expressed in female survival and fecundity. The males need only be retained until they have mated once or twice. They can be discarded thereafter (about 10–15 days after emergence). In *G. morsitans*, the first ovulation takes place about 9 days after emergence. It has been calculated that *G. morsitans* females need not be kept in the colony longer than 108 days. Older, less

productive females can be removed without affecting the colony's puparial yield. During those 108 days, 10 larvae can be expected from any one female.

All the statistics measuring the performance of the colony are based on the original number of females in it. From longevity and fecundity data, it is easy to calculate the mean number of puparia/female/month or throughout their total 108 days in the colony.

Mortality must be checked weekly, to allow calculation of the percentage daily mortality. This should not rise above 1.5%, since a daily mortality of 2%, if maintained for long enough, will destroy the colony. More than 50% of the total number of puparia produced are surplus and can be made available for other purposes (Jordan 1975; Itard 1975a).

Besides the quantitative performance of the colony, a constant check on the quality of the colony output is necessary. This is most conveniently done by determining individual puparial weight. The tsetse reared in the laboratory should resemble wild ones as closely as possible. This is particularly important if the flies are being produced for sterilization and release in nature, where they will have to compete with naturally present tsetse. Females suffering from nutritional stress produce lighter puparia (Mellanby 1937; Boyle 1971). From small puparia, small flies emerge. During pupation, metabolic energy is derived from the puparial fat reserves. In nature, enough reserves must be left for the teneral fly to survive until it finds a suitable host for its first blood meal. Detailed studies on this subject have been undertaken by Bursell (1960c), Rajagopal and Bursell (1965), Phelps (1973), and Phelps and Clarke (1974). Also, field observations show that during hot and dry seasons, selection eliminates small individuals from *Glossina* field populations (Bursell and Glasgow 1960; Glasgow 1961). This selection expresses a shortage of nutritional reserves in small flies. By comparing the size of young, field collected, *G. morsitans* males with those emerging in the laboratory from field collected puparia, Phelps and Clarke (1974) found that, in nature, smaller flies were selected against for about 7 months of the year. Besides female longevity and fecundity, the puparial weight is thus of critical importance.

Tsetse fed on animals and kept under optimal conditions produce puparia of comparable weight to those collected in nature. This had not always been so with flies reared *in vitro* (p. 127, 136).

History of *In Vitro* Feeding

Tsetse can only take a blood meal after the proboscis has actually pierced a membrane. Thus, for *in vitro* feeding, the membrane should have at least some of the attraction exercised by the skin of a favoured animal host. At first, different membranes of animal origin (skin of rats, mice, guinea pigs, as well as bats' wing or fibrine membranes) were tested (Yorke and Blacklock 1915; Roubaud 1917a; Lester and Lloyd 1928; Cockings 1961; Southon and Cockings 1963; Kimber and Harley 1965; Moloo 1971; Rogers 1971; Rice et al. 1972). However, these membranes could be used only for feeding a small number of flies. Therefore, an inexpensive, simple and durable artificial membrane was needed for economical large-scale tsetse rearing.

Only recently has it become possible to satisfy some of these requirements, and to establish a colony based on an *in vitro* feeding technique. The first step was the development of an agar-agar membrane incorporating a terylene netting, resting on stretched parafilm itself supported by grooved glass panes over which the desired blood is poured. This system proved practical for feeding *G. austeni* and *G. morsitans* (Langley and Maly 1969; Langley 1972; Mews et al. 1972). Studies of *G. morsitans* fed via an artificial membrane were also reported by Nobre and Santos (1970) and Oliveiro and Nobre (1970). The nature of the membrane was not defined

by these authors, but Azevedo et al. (1968) had earlier reported upon initial successes with agar and fibrin membranes in their Lisbon laboratory. Azevedo et al. (1970) indicated that final selection fell upon agar spread over four gauze layers and cooled.

A major step was afterwards taken towards maintaining tsetse without living hosts, when a colony of *G. morsitans* fed a mixed regime developed satisfactorily. These flies were fed five times weekly on defibrinated bovine blood through the membrane; and once a week on the ears of rabbits (Mews 1972; Mews 1975). However, the agar-agar/parafilm membrane had to be prepared daily and could only be used for a short time. This was because it dried rapidly, and became unattractive to the flies. Another disadvantage of the agar-agar gel, particularly when in contact with blood, was the proliferation of bacteria, some of which proved pathogenic when ingested by the flies (Bauer and Wetzel 1975). Recently, a silicone membrane was developed that is attractive to *G. morsitans.* It is biologically inert, easy to clean and sterilize, durable, and inexpensive (Bauer and Wetzel, unpublished). This artificial membrane is now used for *G. morsitans* in Seibersdorf and Langford. It can also be used for *G. austeni* and, with some modification, for *G. palpalis.*

Present Status of *In Vitro* Feeding

For a long time, puparia produced by flies fed *in vitro* were about 10% lighter than those produced in animal fed colonies. It was observed that flies fed *in vitro* took 10% less blood than those fed on vertebrates (Langley et al. 1976). This was claimed to be the reason for the lower puparial weight. Also, on a mixed feeding regime the puparia produced were lighter than those from control flies fed on rabbits (Mews 1972, 1975). Until late 1973, no self-supporting colony had been established on a strictly *in vitro* feeding regime, and puparial weight decreased in succeeding generations.

Since then, though, puparia of consistently higher mean weight than those obtained with the mixed regime have been produced on a strict *in vitro* feeding regime. This improvement resulted from a series of measures, the effects of which cannot yet be separated. These measures included the introduction of the new membrane, a higher feeding temperature, reduced handling, and the use of defibrinated horse blood collected once a week. Horse blood thus treated replaced defibrinated bovine blood, which, at least for *G. morsitans,* had never given satisfactory results. It is known that colonies maintained on blood from different animals produce puparia of different weight. This observation implies that besides blood uptake during *in vitro* feeding, blood quality is also of great importance. In Langford, defibrinated pig-blood (collected twice weekly) gives results similar to those obtained with flies fed on goats (Mews et al. 1976a). With *G. morsitans,* excellent results can also be obtained with rabbit blood fed exclusively through membranes. Thus, at least for this species, *in vitro* feeding is fully comparable to feeding on living animals.

Ca-oxalate, Na-citrate, ethylenediaminetetra-acetate, dicumarol, hirundine, and heparine were tested as anticoagulants to prevent defibrination during collection and storage of blood. All of these, except Na-heparine, were toxic to the flies or could not prevent coagulation long enough. However, with 150 I.U. Na-heparine/100 ml, blood can be stabilized for at least 1 week. For maximum effect, heparinization of blood has to be carried out step by step as the blood is being collected.

Methods for ensuring blood preservation must be developed before we can become completely free from the need to collect fresh blood regularly. In this regard, initial trials with freeze-dried blood failed. Deep-frozen blood on its own proved less satisfactory than fresh blood. However, the difference in puparial weight can be made up by certain additives. Cur-

Equipment of in vitro membrane feeding technique used at FAO-IEA, Vienna (H. Wetzel).

rent experiments in Vienna show that the mean puparial weight obtained when flies are fed *in vitro* can be increased by adding to the blood, proteins or certain protein fractions (Wetzel 1974). A completely artificial diet has yet to be developed.

In Vitro Feeding Technique Used at IAEA

One of the main stimuli inducing probing through a membrane is the surface temperature (Dethier 1954; Langley 1972). Until recently, this was regulated by a circulating warm-water system that heated the membrane (Mews et al. 1972). Inexpensive electric warming plates have subsequently been developed. Each warming plate is essentially a hot plate, 45.5 × 45.5 × 2.5 cm, consisting of resistance wire wound around a sheet of asbestos and subsequently encased in aluminium. A thermostat is used to regulate the temperature (Wetzel et al. 1975).

Glass panes with straight parallel grooves are placed on the heating plate. The glass is flamed twice before the blood is poured over it. The glass panes are now replaced by 0.15-mm thick polypropylene sheets with a vacuum formed structure. The sheets are very inexpensive and can be discarded after a single use. However, if the need arises the polypropylene sheets can be reused after cleaning. They also have the advantage of not being easily broken. Just before use, the membrane is immersed for a few minutes in hot water, followed by a similar immersion in lukewarm water. The water is shaken off, and the membrane (still slightly wet) is placed over the blood pool on the sheet. The blood spreads out very evenly between the glass pane and the overlying membrane, due to capillarity and the pressure of the

fly cages. The surface temperature of the membrane is 37–38 °C. Tsetse are generally left to feed for 10 minutes (15 for *G. palpalis*). The type of cage is important because a uniformly close contact between its netting and the membrane is necessary. Plastic cages with tightly stretched terylene netting on two surfaces are most satisfactory (Mews et al. 1972). In the standard 12.5-cm diameter × 5-cm high cage, 15 female flies are kept; in the newer 21-cm diameter cage, 50–60 females. In still larger cages, fecundity is reduced. When the same membrane is used two or three times, the surface becomes soiled with faeces (easily removable with soft paper tissue). The frequency of using a given membrane and blood pool depends mainly on their asepsis. Great care must be taken to prevent bacterial infection.

Normally, one membrane is used for four to six batches of flies. In laboratories specialized in *in vitro* feeding, more than 15 membranes are simultaneously in use. The speed of feeding, therefore, not only depends on logistics and available space, but also on other work carried out during feeding. Routinely, from 60 to 200 females are kept together in a feeding unit. In such a unit, the flies are of the same age and their performance is recorded as a group. The larvae drop through the cage netting onto a tray or into any suitable collector where they can pupariate (the collector can be designed to assemble the puparia from several units). Puparia are gathered, counted, and recorded while the adults are feeding at the membrane. Once a week, the cages are checked for dead flies, which are removed. The handling of flies, cages and puparia, and feeding of units, may be modified to meet special circumstances, e.g. when the performance of the flies is not up to standard. The fly cages are on specially designed trollies or racks. From the breeding facility they are taken to the adjoining feeding room, both of which are environmentally controlled. Diffused light is recommended during feeding.

Cleaning all implements involved in the feeding procedure is a very important part of the *in vitro* technique, and is done in a third, separate room. Any blood soiling the heating plates or table must be removed. As well, contact between the cages and blood (through defects in the membrane) must be prevented. After feeding, the membrane and the underlying glass pane adhering to it, are put into a basin of cold water. The glass pane and the membrane are then peeled apart, the blood on them being carefully washed off under running water. The glass panes are stored dry until used the next day. The membrane is washed twice in boiling water and hung up for drying. The next day, before use, the membrane is put into hot and then warm water as described above. The recently developed polypropylene sheets, which can be discarded after a single use, greatly reduce the danger of bacterial infections.

This technique is satisfactory for *G. morsitans*. Its adaptation to *G. palpalis*, *G. austeni*, and *G. tachinoides* is in progress.

Bacterial Infection in the *In Vitro* Feeding Technique

As long as blood remains the basic nutrient for feeding tsetse flies, the danger of sepsis is constant. When aseptic blood is poured onto the silicone feeding sheet, its temperature (36–37 °C) is ideal for bacterial growth. Many airborne, adventive bacteria can proliferate on such a protein-rich medium and be taken up by the flies. Ensuing infections can harm the flies through various forms of pathogenicity. Some bacterial species are pathogenic through a system of proteinase enzymes. Others, through autolysis, release an endotoxin that may kill insects even though living bacteria are no longer present. Disrupting the gut epithelium, the toxin provides a portal of entry into the haemocoel for other intestinal-tract microorganisms. The resulting septicaemia kills the insect. Flies dead from starvation are easily recognized by their absolutely flat, paper-thin abdo-

men, whereas those dying from bacterial infection show a bloated "black abdomen" swollen with noncoagulated, black or red blood from which bacteria can be isolated (Bauer and Wetzel 1975).

A major incident of bacteria-induced mortality among tsetse flies fed through membranes was studied by Bauer and Wetzel (1975). Five bacterial genera (*Aeromonas, Haffnia, Flavobacterium, Micrococcus* and *Pseudomonas*[16]) were isolated from dead or dying tsetse. Swabs taken from all components of the *in vitro* feeding technique yielded the same bacterial genera. Subsequently, the complete infection cycle in the *in vitro* feeding regime was experimentally demonstrated. In this cycle, bacteria in the blood pool are taken up by the tsetse flies and after 3–5 days reinjected with the saliva into the blood. Furthermore, transmission through the puparial stage to the following generation could be shown (Bauer 1974).

Among the bacteria isolated, *Pseudomonas* sp. was found to be pathogenic on its own even when fed in low concentration. (Unfortunately, since this pathogen does not form spores, it cannot be considered as a candidate for microbial control purposes.)

These findings underline the importance of cleanliness in *in vitro* feeding. Although the laboratory probably cannot be maintained in a completely sterile state, it obviously must be kept as clean as possible. Replacement of the agar/parafilm membrane by a silicone membrane and the glass panes by a thick silicone sheet were important steps in this direction. The procedures for maintaining asepsis of the silicone membrane and supporting sheet have already been described. In addition, vials, flasks, and any other equipment containing blood must be dipped into a water bath to soften any coagulum and then carefully washed.

As a general rule, contact between the flies and protein material where bacteria are developing must be avoided. In this connection, particular care should be taken to prevent movement of personnel and material from the washing to the feeding room while feeding is in progress.

In a mass-rearing facility with an output of 80–100 000 female flies, it is advisable to have the flies divided into subcolonies of 10–15 000 individuals. Each subcolony should be handled completely separately by different personnel using their own equipment.

Treatment Against Bacterial Infection

A regular output cannot be maintained if the daily mortality rate in the colony exceeds 1.5% for very long. Complete asepsis being difficult and expensive, different treatments were tested. The following treatments proved effective against three levels of bacterial infections.

1. Suspected or incipient infection, i.e. daily mortality first reaches 2%: the infected or suspect flies are fed for a week through a newly prepared membrane on aseptic blood. At each feeding, the blood pool and membrane are used for this particular batch of flies only, feeding being as rapid as possible.

2. Slight infection, i.e. daily mortality up to 3%: (a) fresh blood collected daily or every second day and preserved with Na-heparine is fed to the flies. Each blood-pool and membrane is used for only one lot of flies. This must be done at least for 1 week and until the mortality decreases; and (b) flies are fed *in vivo* for three or four successive days.

[16] The only genus common to this list and a list of eight bacterial genera — *Enterococcus, Corynebacterium, Bacillus, Proteus, Enterobacter, Citrobacter, Bacterium,* and *Pseudomonas* — isolated from *G. morsitans* and blood fed through membranes to them by Nobre and Santos (1970). Further emphasizing the saprophytic nature of such infections, similar isolations by Oliveira and Nobre (1970) yielded representatives of five more bacterial genera — *Staphylococcus, Providence, Serratia, Alcaligenes,* and *Chromobacterium* (ed).

3. Heavy infection, i.e. daily mortality exceeds 3%: the appropriate therapeutic drugs are administered through *in vitro* feeding on three successive days (see below).

The rationale for feeding aseptic blood through a sterile membrane to sublethally infected flies (1 and 2 above) is that such flies can survive if no additional infection occurs. Fresh, nondefibrinated blood or blood taken by the fly directly from an animal has a bacteriostatic effect.

Very rarely is there a need for treating the entire colony in cases of incipient mortality. This is because flies of the same age are kept as units. The first increase in mortality can thus very often be detected in one or a few units and before six age group periods are completed (54 days after emergence). Early detection depends on daily checking for mortality, death from starvation being easily differentiated from bacterial disease.

As a rule, young units should always be fed before old ones to prevent their contamination by sublethally infected older flies. Usually, sublethal infection occurs in units fed from the same blood pool. Those beginning to show increased mortality should be fed first.

Feeding flies on living animals is an easy and effective way of controlling slight infections. However, with this therapy a rapid increase in mortality sometimes occurs during the first few days. The explanation for this is that the blood intake from a living animal is larger than through a membrane. If infection has slightly damaged the gut epithelium through a proteolytic enzyme, a large blood meal taken from the animal causes the epithelium to burst, and the fly dies within a few hours. For this reason, heavily infected flies cannot be cured by feeding on animals.

More than 3% daily mortality (3 above) represents a serious drain on the colony, for it reduces puparial production and, consequently, the number of flies in the next generation. Since bacteria are transmitted to that generation via the puparial stage, the first step in controlling such a level of disease should be the destruction of puparia emanating from the heavily infected flies before or during actual therapy. Treatment depends on effective chemotherapeutic agents, which should always be readily available.

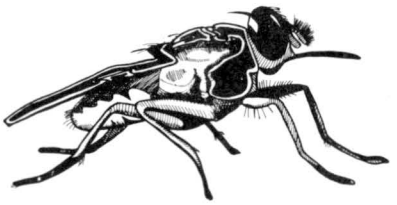
Nobre and Santos (1970) reported that the addition of tetracycline (0.25 mg/ml) to blood used for membrane-feeding reduced the subsequent mortality rate, and the number of bacteria-positive cultures obtained from the internal organs of *G. morsitans* killed after such feeding. In Vienna, sulfonamides and antibiotics (oxytetracycline, neotetracycline, chloramphenicol, penicillin, and rimactane) were tested. Among these, only rimactane was toxic to the flies. After screening for bacterial sensitivity, any of the other drugs should be administered at the rate of 20–25 ppm in the blood pool.

To date, most of the bacteria causing serious mortality in tsetse fly colonies have been found to be sensitive only to tetracyclines. This is unfortunate, for following treatment with these compounds fecundity is reduced (Wetzel and Bauer 1975). After the first larviposition the reduction in fecundity of tetracycline-treated flies is about 50%; after the second, (28th day after emergence) 30%. From the deposition of the third or fourth larva, and up to the end of the 10th reproductive cycle, a very slight reduction or none at all occurs. A possible reason for this reduced fecundity is damage to the symbionts (Hill et al. 1973). According to preliminary investigations by Nogge (1974), it seems that in tsetse, as in other blood-sucking insects, the symbionts play a role in B-vitamin metabolism. One or several of these B-vitamins may regulate oogenesis.

Bacterial infections in tsetse colonies can be effectively controlled with appropriate treatment regimes. However, in many cases it will be necessary to combine some of these regimes. As already indicated, success depends on early detection of each increase in mortality. Bacterial monitoring can be carried out by taking swabs from the blood remaining on the membranes after feeding, and from the hands of personnel. These swabs should then be incu-

bated on nutrient agar-agar. These tests help clarify sources of bacterial infection, and provide a check on the degree of asepsis of the system (Bauer and Wetzel 1975). It is also advisable to test the sensitivity of isolated bacteria to certain drugs, which can then be kept on hand for therapeutic treatment.

It is evident from the above description that rapid progress has been made during the past 3 years in developing *in vitro* feeding techniques for tsetse-fly rearing. There undoubtedly will be many more problems when *in vitro* fed colonies exceed 50 000 producing females. However, the fact that successful *in vitro* feeding techniques have been developed is very encouraging. Likewise, adding various nutrients to defibrinated blood with beneficial effects augurs well for the eventual development of a completely synthetic diet for tsetse flies. Although tsetse flies never will be reared as simply or cheaply as some other Diptera (e.g. mosquitoes), the increased efficiency of the *in vitro* feeding technique will greatly decrease the cost of rearing them. — H. Wetzel.



Mass Production of Parasitoids, Parasites, and Pathogens

Insect Parasitoids

In considering the use of insect parasitoids for the biological control of tsetse flies, attention must be directed toward parasitoid procurement in numbers sufficiently large to allow their use on a suitable scale to achieve a reduction in the numbers of *Glossina*. There are two principal ways in which control may be attempted: (1) by establishing by means of a few releases a parasitoid species that does not already occur in an area where control is required; and (2) by releasing periodically within an area a large number of local or imported parasitoids with a view to increasing parasitism and to reducing the host population to a low level for a limited time. Additional releases of parasitoids would be made as the pest population warranted.

Of these methods, the former has obvious advantages. A parasitoid once newly established in an area hopefully becomes a permanent component of the complex that attacks *Glossina*, increasing the destruction of tsetse and reducing their numbers without the necessity of a large number of repeated releases. According to its biotic characteristics, such a parasitoid will be able to spread from the release points over an area limited only by its ecological requirements. The initial numbers released to achieve establishment are not usually very large if the parasitoid is in fact ecologically suited to the area and to the behavioural patterns of the host species. In the second method, there is no aim at permanent establishment of a new addition to the parasitoid complex. Instead, sufficient individuals of one or more parasitoid species are released in such numbers and at such times as to cause an immediate impact on the numbers of *Glossina* emerging. An additional beneficial effect may be the reduction of pest numbers in the next generation. This entails the use of much greater numbers of parasitoids than in the first method, and their careful distribution over the area where control is desired. In both methods care must be taken to make releases at a time when host puparia, the only stage of *Glossina* that parasitoids can attack, are present in maximum numbers.

Both of these methods necessitate the provision of adequate numbers of parasitoids either as adults or as parasitized puparia. In some instances, it may be possible to field-collect sufficient parasitoid material for use in another area, particularly when comparatively small numbers are required for a single introductory release of the first type. However, in all attempts of the second type, especially with *Glossina*, it will be necessary to breed the parasitoids required for the releases.

The parasitoids recorded from *Glossina* and information on their biology and distribution have already been discussed. In the few instances where biocontrol has been attempted, attention has been paid to the mass-breeding and release of *Syntomosphyrum*. These particular examples indicate the need to be very careful when selecting a species of parasitoid for mass propagation. A parasitoid should be selected as a candidate species for a biocontrol attempt because of its: (1) ecological adaptation to the environment it will encounter; (2) high searching ability that will enable it to find host puparia, which may be scattered over a wide area; (3) ability to increase rapidly in numbers when hosts

are abundant; (4) ability to maintain its population when hosts are scarce; and (5) ability to survive periods of abnormally adverse weather. It may not be possible to find a parasitoid with all of these advantageous characteristics. In that case, the species that most closely approximates the "ideal" is the best for any trial and, hence, should be selected for breeding.

The suitability of a parasitoid in any of these aspects can only be determined by extensive experiments. This necessitates the availability of large numbers of the organism and, therefore, some degree of mass breeding. Even determination of the most promising parasitoid for a biological control attempt demands a mass breeding technique.

How these techniques are developed will be determined by a number of factors, the most important being the biological characteristics of the species involved. The facts that in *Glossina* the puparium is the only stage that is attacked by parasitoids, and that tsetse puparia are comparatively very expensive to produce, makes them singularly inappropriate for use in the mass breeding of parasitoids.

The most promising of the *Glossina* parasitoids for use in biocontrol are the bombyliids and mutillids, the individual species of which do not occur throughout the range of *Glossina* in Africa. In fact, no mutillid has been recorded from west Africa. The inevitable conclusion is that attempts should be made to establish them there. In this connection, it is obvious that host suitability tests must be carried out to ensure that the individual parasitoid species will in fact attack the particular target species of *Glossina*.

With bombyliids, great difficulty has been experienced in breeding them at all, let alone in large numbers. The females are strong, rapid flyers, and mating and oviposition only occur on the wing and in full sunlight. It was only recently that *Villa brunnea* Becker was induced to oviposit in cages, the eggs being deposited in specially prepared "cracks in the soil" (Du Merle 1970). These cracks are organized in such a way that the eggs then drop into vials and are kept there until they hatch. The resulting "planidium" larvae are then placed on *Thaumetopoea pityocampa* (Schiff.) puparia into which they bore. They subsequently complete their development endoparasitically.

It has been noted in a bombyliid parasitizing *Glossina* in west Africa that the distribution of parasitoid larvae over a number of host puparia is such that superparasitism never occurs. This indicates that, prior to boring into a puparium, the young bombyliid larva selects only nonparasitized, healthy host larvae. If this characteristic is general, it means that a great economy of both bombyliid eggs produced and host puparia used can be achieved in any mass breeding attempt. However, a great deal of work will have to be done to develop suitable cages for mating and oviposition, egg-collecting, hatching, and penetration of host puparia before it will be possible to develop suitable mass breeding methods for bombyliids. Bearing in mind their importance as *Glossina* parasitoids, with high degrees of parasitism in many instances, it is essential that this basic investigative work be started as soon as possible.

Some work has already been done on breeding mutillids. Lamborn (1925) bred *Mutilla glossinae* Turner in small numbers. He succeeded in doing so using both *Sarcophaga* sp. and *Glossina* sp. puparia as hosts. In view of their general importance as *Glossina* parasitoids and the apparent lack of difficulty in breeding them, attention should be directed toward the development of mass breeding techniques. Even though their developmental stages are of rather long duration, this should not deter efforts. This is especially the case if it can be shown that female mutillids have a high searching ability and are long-lived under adversely dry climatic conditions, enabling them to discover dispersed *Glossina* puparia in savannah areas. After the bombyliids and mutillids have been studied, it may well be found that other *Glossina* parasitoids warrant a similar approach.

In any consideration of mass breeding

we are faced with the expense involved in using *Glossina* puparia as hosts. One of the principal avenues of research would be to obtain alternative hosts suitable for mass breeding different parasitoids. Lamborn (1925) bred *Syntomosphyrum* on *Sarcophaga* puparia, Nash (1933a) bred them on *Chrysomyia* sp., and Roubaud and Colas-Belcour (1936) used *Musca domestica* L.: Saunders (1960a, b) bred the above species and *S. albiclavus* on *Lucilia sericata* Mg. and on several other blowflies (Cyclorraphinae). Similar investigations should be carried out to determine which additional alternative host puparia might be used in breeding bombyliids and mutillids with a view to obtaining an inexpensive, readily mass-produced host. The very wide range of possible hosts available includes species of *Musca, Lucilia, Sarcophaga, Ceratitis, Dacus* and *Drosophila*. For all of these, detailed techniques have already been developed for inexpensive mass production.

Another possibility is the use of muscid parasitoids (not always known from tsetse-ridden areas of Africa) against *Glossina*. There are a number of these, including species of *Spalangia, Muscidifurax raptor, Pachycrepoideus vindemiae, Stenomalus muscarum* (Pteromalidae), and the staphylinid beetle *Aleochara taeniata* (see Legner and McCoy 1966; Legner and Olton 1968). Mass production methods for these have already been developed. The details of the methods must be adjusted to suit both the parasitoid and the puparia of the host species being used. If houseflies, for example, are being used as hosts, adults can emerge, mate, and oviposit in wood framed, screen cages (approximately 25 × 20 × 10 cm) with sliding plastic front panels. Cotton-wool pads soaked in skim milk and changed daily serve both to provide adult food and oviposition sites.

Pads bearing eggs are removed from the cages and placed on chicken feed moistened with skim milk in 1-litre oil tins, which are then covered with screened lids. When the fly larvae are almost fully grown, the contents of several tins are dumped into 0.25-cm mesh, screen-bottomed trays placed over a wooden tray containing presifted chicken feed. Fully fed larvae wriggle through the screen and drop into and pupariate in the wooden tray. A day or so later the contents of the trap are sieved, the housefly puparia being retained by a 0.25 cm screen mesh. The puparia obtained are then used both to maintain the housefly stock and in parasitoid breeding programs. In some areas, programs for the abatement of muscid flies in poultry runs depend in part on periodic releases of hymenopterous parasites. Housefly eggs are seeded into large plastic tubs containing a special housefly rearing medium produced commercially by livestock-feed manufacturers. When the larvae are fully grown the tubs are flooded, forcing the larvae to crawl out to find suitable pupariation sites. They drop from the tubs into funnels or sloping troughs leading to large plastic tubs, where pupariation occurs.

Housefly puparia can be exposed to adult parasitoids *en masse* in a variety of containers depending on the species being bred, numbers required, and local availability of containers. At the Commonwealth Institute of Biological Control (Trinidad), for example, 5000–10 000 puparia were placed with 500–1000 adult parasitoids in one gallon plastic ice-cream containers with a fine-mesh screen placed over the top. High levels of parasitism were obtained. In the United States, "stinging-bags" consisting of cloth sacks closed by means of a zipper, and containing some 100 000 or more puparia and 5–10% as many parasitoid adults, are used for mass breeding. The sacks are laid flat on benches and turned periodically, to expose the puparia to maximum contact with the parasitoids. The houseflies emerging several days ahead of the F_1 parasitoids, are returned to the housefly breeding cages. The empty puparial shells are then blown off, leaving only parasitized puparia. These can be held for adult parasitoid emergence, or distributed in the field.

Each species of parasitoid will require particular techniques to obtain the optimum and most economic production of

units fully suited to field survival on the scale required. Questions of stress (Sonleitner 1964) and perhaps heterogeneity (p. 181) are pertinent. It is, however, evident that with some parasitoids there is no limit to the numbers that can be economically produced. Obviously, experiments must be carried out to determine which are the most suitable hosts for use both for these parasitoids from muscid hosts and, also, for the *Glossina* parasitoids. This alone may require breeding in large numbers, using *Lucilia, Dacus, Drosophila,* and other hosts. Possibly, it may be necessary to develop mass breeding techniques for other host species whose puparia are more acceptable to the *Glossina* parasitoids. For each species of parasitoid, mass production techniques adapted to the numbers required must be developed and modified in accordance with the way in which the resulting progeny are to be used in the field.

Because some of the known *Glossina* parasitoids warrant introduction into areas of Africa in which they do not already occur, the development of breeding methods for bombyliids and mutillids merits high priority. Others may warrant mass production and release in the manner that *Syntomosphyrum* was used. Here, though, it is essential to determine *a priori* the ability of the species selected to operate effectively under the ecological conditions in which control is required, before mass production for release is undertaken. Simple mass breeding techniques for some of the muscid parasitoids that might be tried against *Glossina* are already well-known. Here, careful selection of the parasitoids is necessary to determine: (1) if they will, in fact, successfully parasitize the desired *Glossina* puparia in the field; and (2) if they can operate successfully there under the exact ecological conditions both of climate and host dispersal in which these *Glossina* puparia occur.

Initial testing of some parasitoids and trial breeding will have to involve the use of *Glossina* puparia. Limited supplies of several species for experimentation can undoubtedly be made available from existing production units in several places, e.g. Austria, France, and the United Kingdom.

Microbial Pathogens

The mass production of effective microbial agents for the biological control of a target insect is based upon the assumption that the microbe can be propagated under conditions of maximum control. Mass propagation of a microorganism can be best attempted by using: (1) the inoculation, or another mode of infection, of laboratory reared insects; (2) the growth of the agent with or within a susceptible host or other cell line(s); or (3) the growth of the pathogen in pure culture on media that may or may not be well-defined. Because of the large number of organisms required, large-scale laboratory or industrial-scale fermentation is the preferred production method whether pure or two-member cultures are used. In addition to the quantities of a given biocontrol agent required, the producer must be in a position to give assurance as to the quality of the product with regard to several criteria, including: virulence, stability, and safety. To assist in meeting these criteria, those responsible for mass production should bear in mind the following questions: (1) What is the objective for the use of the pathogen? (2) What is the dosage required to achieve that objective? (3) What are the best techniques for evaluating the ecological impact and effectiveness of the pathogen? (4) Does sufficient field and laboratory information exist to permit the above evaluation? (5) What is the required method of delivery for the pathogen? (6) Is the final envisaged pathogen preparation (wet or dry) compatible with the delivery technique and the activities of the pathogen? (7) How long and under what conditions will the pathogen remain active (or dormant) within the intended environment? (8) What is the route of host infection? (9) How genetically stable is the pathogen?

Sporangia (arrows) containing sporangiospores of the fungal tsetse pathogen, Absidia repens (× 2500). The culture was grown for 18 days on potato-dextrose agar at 25 °C, and provided by C.W. Hesseltine (R. Nolan).

(10) How readily can laboratory developed strains of the microbe be differentiated from naturally occurring strains of the pathogen? and (11) What conditions, including the possible use of stabilizing agents, are necessary for storage of the pathogen with the maintenance of virulence?

Keeping in mind the need for the producer to provide an effective and safe product, the growth of the agent under well-defined physical and chemical conditions implies an improved quality of the pathogen as the producer assumes greater quality control of the substrate. However, in the harsh light of financial reality, the use of glucose, glycine, tissue-culture grade lactalbumin hydrolysate, and other high-purity or complex, synthetic reagents in large-scale fermentation is economically prohibitive. The determination of the nutritional requirements of the organism and of any mutant strains is conducted during the early phases of the study. This will become part of the overall characterization profile of each strain and be used in the screening of less well-defined media components for undesirable attributes (for example, the possibility of a toxic phosphate level or NaCl level, as in the case of acid (HCl) hydrolysates of proteins, which are neutralized with NaOH).

In the case of work with *Glossina*, we have rejected the possibilities of obtaining large numbers of pathogens through: (1) the collection of individual hosts infected with the pathogen as a result of natural epizootics; (2) the establishment of the pathogen in a natural population of the host and the subsequent collection of the diseased hosts; and (3) the infection of wild hosts that have been collected in the field and then maintained for a short period in the laboratory. One of the essentials in any biocontrol endeavour, howev-

er, is the ready availability of colonies of different strains of the host for testing the pathogenicity, route of infection, and mode of action of potentially valuable microbial strains. These known "tester strains" of *Glossina* spp., growing under controlled conditions, represent a vital component in the bioassay system employed for pathogen evaluation both in the initial stages and in later use with the formulated product. The determination of the virulence of a pathogen and of its safety for target and for nontarget organisms depends upon the use of quantitative bioassays using not only the host "tester strains" but also other selected biological components of the host's natural environment. These latter components may vary markedly within the large geographical range of the genus *Glossina*, which covers roughly one-third of the continent of Africa.

Specific Pathogens

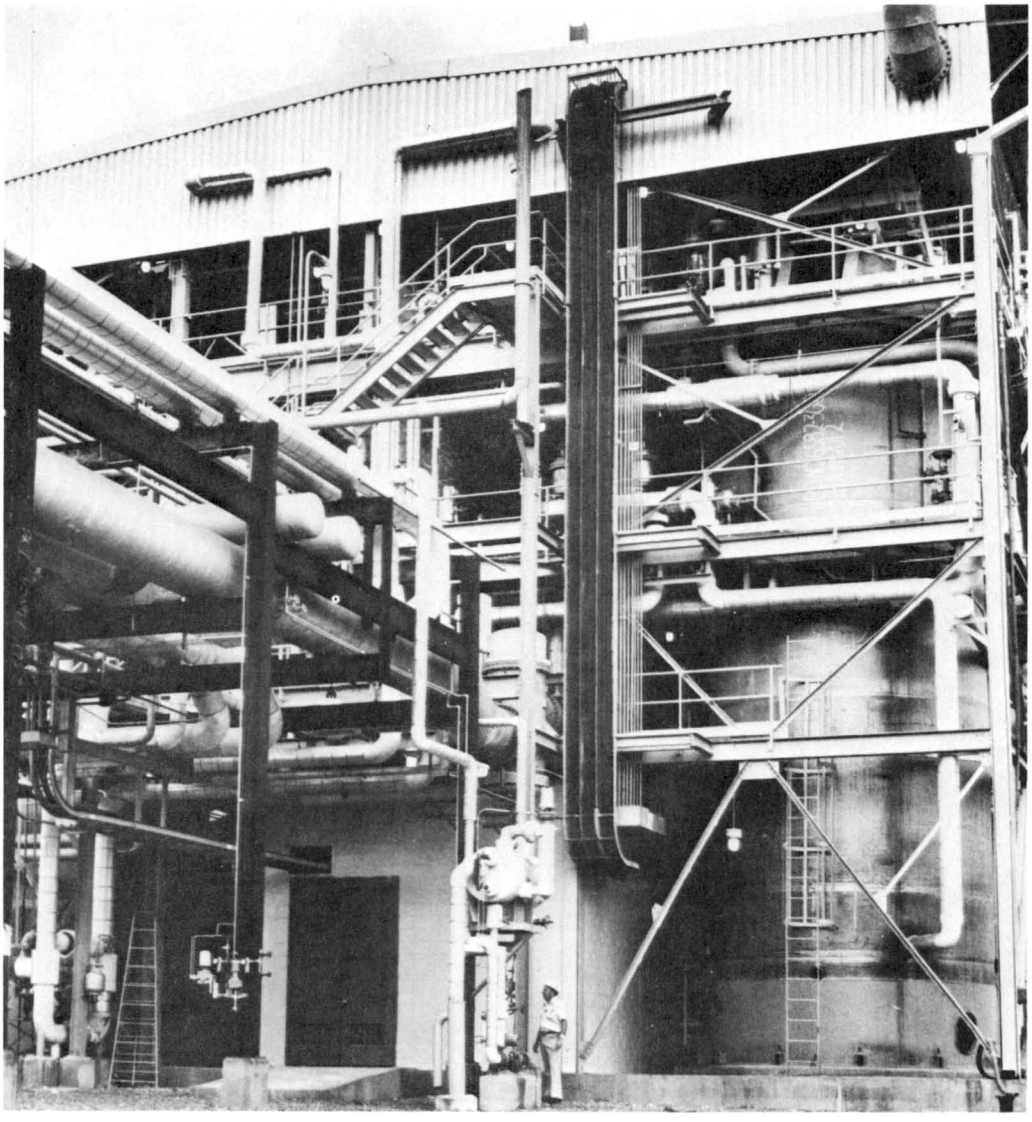
Recent reviews of the literature (see Pathology and Nematode Parasitism) and Roberts (1976), indicate that of the organisms that have potential, and for which isolates are available, the fungus *Absidia repens* (Vey 1971) is a promising pathogen of the puparial stage. *Pseudomonas* sp. and *Aeromonas* sp. (Bauer 1974) appear to have promise for development as pathogens of adults. Representatives of the fungal genus *Entomophthora* need to be reevaluated under proper experimental conditions before they are ruled out as potential biocontrol candidates for *Glossina*. The previous work with *Glossina* and *Entomophthora* has been far from definitive (Roubaud 1911; Vanderyst 1923).

A. repens has an advantage in that it could be readily produced at the commercial fermentation level (150 000 litres and larger) by growth on a medium composed of 2% sucrose plus 0.25% corn steep liquor (personal communication from Dr C.W. Hesseltine, USDA, Peoria, Illinois to R.A. Nolan, 1 February 1974), which has been used to grow other closely related organisms. Representatives of the genus *Absidia* are common components of the soil microflora. They are able to reproduce asexually by means of sporangiospores that aid in dissemination, and can also reproduce by sexual mechanisms to form a dormant, resistant zygospore (see Hesseltine and Ellis 1966). The strain of *Pseudomonas* used by Bauer (1974) has not been fully characterized. However, representatives of this genus are chemoorganotrophs that, generally speaking, lack highly specific growth factor requirements, and can develop on a wide variety of organic compounds. Neither has the strain of *Aeromonas* used by Bauer (1974) been fully characterized, although representatives of this genus are also chemoorganotrophs. They hydrolyze casein; therefore the use of commercial-grade casein hydrolysate in fermentation-level growth is a possibility. The optimal pH ranges for the growth of species of *Pseudomonas* (pH 7.0–8.5), with no growth at pH 6.0 or below, and of *Aeromonas* (pH 5.5–9.0) (Buchanan and Gibbons 1974) indicate that they could quite likely metabolize and be pathogenic in the midgut environment of a tsetse fly, assuming a given strain possessed the necessary toxins and/or enzymes. The midgut has a pH range of 6.3–6.9, if the values for *G. m. submorsitans* can be taken as indicative (Wigglesworth 1929). Although members of the fungal genus *Entomophthora* undoubtedly have a promising future in the area of the biological control of insects, it is as yet impossible to assess their value in an anticipated program with *Glossina*. Much more basic physiological, biochemical, and bioassay[17] work needs to be done with these fungi before relevant large-scale studies are attempted (see MacLeod 1963).

Mermithid Nematodes

Mermithid nematodes are obligate para-

[17] See p. 161.



Enclosed 45 000 gallon (170 000 litre) fermenter located at Arecibo, Puerto Rico (courtesy S.F. Richard, The Upjohn Company, Kalamazoo, Michigan).

sites. Those found in tsetse flies will probably prove host-specific, or at least specific for the genus *Glossina*.[18] Their life cycles and numbers are, therefore, likely to be intimately related to those of the host. The incidence of parasitism of *Glossina* by mermithids has never been reported to exceed 1% (Roberts 1976). This suggests that perhaps these worms may have a low potential as biological control agents. Alternatively, the low incidence in adults may reflect a situation in which the nematode is mainly parasitic on the puparial stage, with adult parasitism serving primarily as

[18] Rubtsov (personal communication 1976) advises that new species of mermithids are being described from *Haematobia* and other relatively near relatives of *Glossina* in the USSR (ed).

a mechanism for transporting nematodes to new sites. Adult parasitism might result from either infection late in puparial development, or infection of the teneral stage at the time of emergence.

It is probable that mermithid nematodes lack potential as biocontrol agents aimed at adult tsetse. Mermithids require very moist conditions. It is thus technically difficult to apply them in spray formulations either to control flies visiting host animals for blood meals, or to treat sites on vegetation where tsetse flies might rest. Adjuvants have not yet successfully extended the lives of nematodes for any appreciable period by preventing desiccation under exposed conditions. Moreover, there are no records of either the successful transmission of mermithids during feeding on higher animals or the successful infection of insects resting on vegetation by mermithid larvae. Any possible potential of mermithid nematodes would best be evaluated by the introduction of the nematode eggs or preparasitic larvae in large numbers into sites where tsetse flies deposit third instar larvae. The appropriate soil habitat could then offer the parasites protection from desiccation until possible penetration of the host.[19]

Several years of intensive research are obviously required before the real potential of mermithids in tsetse biocontrol can be evaluated. If the results are favourable, then advances may follow the direction of the work by Petersen and Willis (1972), for the mass production of mermithid preparasites of mosquitoes. — F.J. Simmonds, R.A. Nolan, J.D. Briggs, and R.F. Myers.

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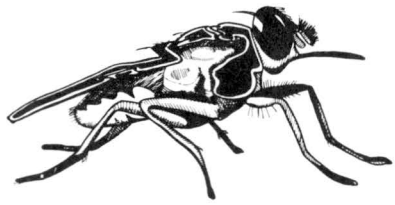
[19] See footnote p. 87 (ed).



Public Health and Environmental Safety

Over the past decade, the increasing demands on world food supply and the need to control vectors of human and animal diseases has resulted in an upsurge in the use of chemical insecticides. Mankind has benefited immensely from the control of vector-borne diseases in many parts of the world, and by the introduction of agricultural practices increasing the yields of food and fibre crops. As the immediate needs for pest and vector control were so obvious and urgent, and the initial success was so overwhelming, the more subtle side-effects and potential long-term consequences were often not considered in advance. The prediction of long-term effects must always be, in part, conjecture, because the facts are only learned through continuous monitoring and observation.

There is nevertheless tangible evidence that some chemical pesticides have had adverse ecological effects, especially where environmentally persistent or long-lasting compounds have been carelessly used. Also, the development of insect resistance to certain chemicals has resulted in the need for increased rates of application. Furthermore, the continuous increases in the price of petroleum (the raw material from which most chemical insecticides are synthesized) have drastically raised pesticide prices and in some cases hampered availability.

We have thus reached a turning point in vector and pest control, where we must consider a more prudent and diversified approach — nowadays termed integrated control, itself a component of the overall technique of pest management. Biological control (whether it be by means of parasites, predators, microbial pathogens, hormones, or pheromones) must eventually play an important part in integrated pest management systems. Synthetic organic chemicals will undoubtedly remain the basis of pest control in the foreseeable future, but they must be increasingly supplemented by alternative methods of control.

The biological control of agricultural and forestry insect pests is further advanced than biocontrol methods for arthropods of public health importance for at least three reasons: (1) the short-term economic implications for agriculture and forestry are greater — consequently, more resources have been available for development of biological control agents; (2) large-scale agricultural and forestry operations are essentially monocultural, but many important disease vectors inhabit diverse, heterogeneous environments, rendering it much more difficult to apply the control agent efficiently; and (3) which is related to (2) most biocontrol agents, including all of the microbial ones, act as preadulticides. In the case of agriculture and forestry pests, the larval stage generally constitutes the problem. The advantage of biological control at this stage is evident.

On the other hand, disease transmission is always effected by adult insects. This fact poses difficulties when evaluating the efficacy of biological control in those species characteristically producing vast numbers of larvae subject to high mortality rates. Yet, in both agricultural and forestry entomology, the point is being reached at which the implications of increased introduction and use of insect pathogens, predators, and parasites must be most thoughtfully considered. We must learn

from our positive and negative experiences with chemical pesticides, and try to avoid the same or similar problems when using biocontrol agents ("third generation pesticides" as they are sometimes called).

Information concerning their health and environmental safety, and long-term effects is replete with speculations, conjectures, and uncertainties, most of which may never be entirely resolved. Investigators in this field must be able to devise an approach that will allow a reasoned evaluation of the possible risks associated with the use of biocontrol agents. To date, most of the limited use of biological control agents against pest insects has involved introductions of highly specific insect parasitoids posing no harm to human health and very little to the environment. Such biocontrol agents are, after all, naturally occurring organisms to which man and his environment are continuously exposed. However, the possibility of a new risk-level being associated with inundative releases of microbial control agents (viral, bacterial, rickettsial, protozoal, fungal, etc.) in new or expanded habitats, must be viewed as an entirely novel circumstance.

Our safety evaluation of biocontrol agents will focus primarily on insect pathogens and parasites, a unique group of biocontrol agents, having the potential for reproduction and infection in nature. To a lesser extent, the release of predators will also be discussed. Insect hormones (or growth regulators) and pheromones will not be discussed. These are chemically defined products. Their safety evaluation should follow closely that used for other chemicals, with special emphasis on such aspects as specificity, use, and residue concentrations. Genetic control (i.e. sterile-male release and other genetic techniques), which are sometimes termed "autocidal" control, will not be discussed here. The safety and rationale of these techniques have been discussed and assessed by others.

The general scheme presented could allow the orderly development of pathogens for pest and vector control. Basically, this program consists of safety testing, limited use, review, and assessments. These sequential phases must be so integrated that a scientific judgement of the probable risk of the next step is feasible. Evaluations of efficacy and safety should proceed simultaneously. Neither a safe agent, which is not efficacious, nor an efficacious one, which is not safe, is of any value.

Predators and Parasitoids of Pest and Vector Arthropods

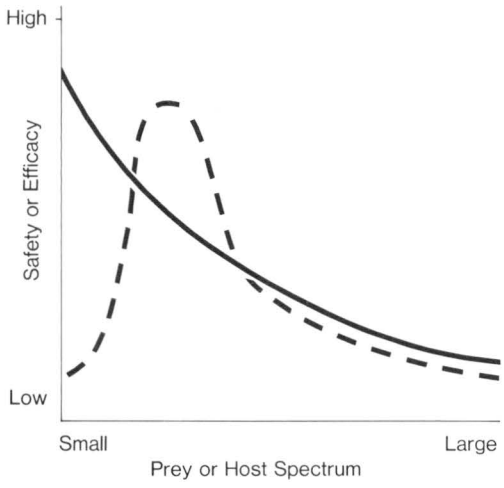
Hazards to Humans

The possible hazard to man resulting from the introduction of pest and vector parasitoids and predators is very low. The possibility that such species may be carriers of human disease is, to say the least, tiny. However, it must be ascertained *a priori*, based on the biology of the predators or parasitoids in question, whether or not it is possible that they could be vectors of human disease. Another adverse effect that may be envisaged is that the introduced parasites and predators could become overabundant, presenting a secondary problem. This eventuality is an environmental issue, to be considered when assessing the environmental safety of species introduced under the proper national entomological quarantines prevailing in the countries concerned.

Environmental Safety

The biology of predators and parasitoids must be known. This not only permits the assessment of their safety, but also is essential to any appraisal of their effectiveness. General food habits and requirements, alternate hosts and prey species, and temperature and humidity requirements affect the performance of introduced biocontrol agents.

Prior environmental and ecological studies are a prerequisite to any such



Relationship of relative safety and efficacy of the host spectrum of parasites and predators (—— relation of safety to host spectrum; - - - relation of effectiveness to host spectrum)

introduction. All conceivable environmental interactions obviously cannot be assessed, but the most significant interrelationships should certainly be studied.

Environmental safety and the effectiveness of predators and parasitoids are closely linked. From the safety aspect, it would be desirable to use biocontrol agents with a very narrow food, prey, and host spectrum. For effective control this may not be desirable, since the chosen predator or parasitoid must be able to survive after decimating its supply of prey or hosts. On the other hand, species that are nonselective as regards host or prey selection, are probably not very effective against a particular target pest.

This situation can be expressed graphically by plotting the projected effectiveness and safety of a parasitoid or predator in relation to its host or prey spectrum. Species selected for control purposes should combine maximum control effectiveness with minimal potential for environmental hazard.

The group termed "parasites and predators" includes a vast number of species from a variety of invertebrates to such vertebrates as fish. Obviously the same safety considerations or standards cannot be applied to all species. In each instance the following questions should be asked: (1) Is human infection possible, or likely? (2) Is uncontrolled spread in the environment, with adverse effects on humans and/or other nontarget species, possible or likely? and (3) Could unforeseen adverse effects be corrected, should they become apparent? Any rationalizations, laboratory tests, or environmental and ecological investigations must be designed and focused to answer these questions.

Pathogens

Entomopathogenic viruses, bacteria, fungi, and protozoa fall within this subheading. There are semantic problems over terms such as "pathogen, pathogenic, parasite, parasitic," depending on the training and background of the investigators concerned. The reason for discussing pathogens separately — although some of them could be classified as obligate parasites (baculoviruses and some bacteria for example) — is that here we are dealing with microorganisms. In considering human health as well as environmental safety aspects, we must exercise considerably more caution. Also, the organisms listed under pathogens are applied in much the same fashion as conventional pesticides. On the other hand, predators and parasites are generally applied by a highly selective release procedure (mermithid nematodes may be an exception).

Major Requirements for Testing Biological Control Agents

The evaluation of a biocontrol agent's usefulness and safety must proceed in an orderly and well-planned fashion. Tests and reviews should be structured in such a fashion that the development of the con-

Table 20. Preliminary scheme for screening and evaluating the efficacy, safety, and environmental impact of biological control agents for control of disease vectors.

STAGE I	STAGE II		STAGE III	STAGE IV		STAGE V
Laboratory A. Identification and characterization B. Assessment against selected target vectors C. Preliminary evaluation of ease of rearing in quantity	*Laboratory* A. Mammalian infectivity tests to ensure safety to laboratory and field personnel	*Results of review of stages I and II*	*Preliminary field trials* Strictly regulated tests under WHO supervision to determine efficacy against disease vectors under natural conditions With the use of larger amounts of biological control agents, methods of application should begin to receive attention	*Laboratory* More detailed tests on mammalian infectivity, using appropriate techniques *Laboratory and field trials* Detailed studies on nontarget range—especially other fauna in representative habitats where stage V trials may be conducted *Formulation* Studies on stability of suitable formulations and delivery systems	*Review of stages I, II, III, and IV by informal consultation group*	*Large scale field trials* To be conducted under WHO auspices. Not presently defined, and will vary according to target vector, habitat(s), mode of application, etc.

trol agent proceeds rapidly, without, however, endangering human health and the environment. Financial resources must be used wisely, checks and balances being applied so that a project can be abandoned in good time if it becomes apparent that the control program is neither effective nor safe. A five stage program,[20] now proposed, is schematically shown in Table 20. It must be stressed that this program is presented for guidance only — the various stages may gain or lose importance depending on the kind of agent to be developed, and on the eventual extent and type of projected use. For example, agents to be used in circumscribed areas, (e.g. small water bodies) will have to be developed through the stages indicated with different points of emphasis from those relating to agents that are developed for large-scale use and/or for use on food crops. It must also be pointed out that demarcation between the stages of development should be recognized as fluid. Certain goals of the next stage should determine or extend experimental designs of tests.

Stage I

The first step in the development of biocontrol agents (especially microbial pathogens) is the isolation of the agent in nature. Biological methods (e.g. host range, and biochemical and biophysical methods) must be used for identification as well as morphology. This very important step varies greatly, depending on the type of agent involved, and certain problems arising in identification are discussed here. Plans are underway as part of the WHO's expanded Medical Research Programme, to establish international and national collaborating centres concentrating upon each type of candidate pathogen, in order to achieve a standardized and uniform characterization. Besides strictly taxonomic identification, growth parameters such as temperature and pH range, substrate or host requirements, and susceptibility to antimicrobial agents must be determined. Such basic information is essential for determining health and environmental safety, and providing reassurance that corrective measures will be taken should adverse reactions occur in persons exposed to the control agents.

A preliminary safety-screening of the prospective control agents must be undertaken in Stage I. Laboratory animals should be exposed to the pathogen by the appropriate route (oral, subcutaneous, intraperitoneal, intertesticular, etc.). This type of screening allows a rapid conclusion about the acute mammalian virulence of a candidate entomopathogen. It will indicate how best to handle the control agent, and how to proceed most sensibly to the more sophisticated and comprehensive safety tests of Stage II.

During this first stage, appropriate bioassays to determine the efficacy of the control agent should be conducted against a range of target pests and vectors. This assessment should be made under conditions of strictly controlled laboratory exposure, so as to gain knowledge about the mode of action of the pathogen. Furthermore, the design of such laboratory studies should approximate the proposed use of the candidate control agent (i.e. aquarium tests for aquatic vector larvae should simulate as much as possible the vector habitat — swiftly flowing water for blackfly larvae or stagnant, low-oxygen conditions for certain mosquito species, or tests in small greenhouses or growth chambers for agricultural and other terrestrial insects).

Essential data can thus be collected on the stability, persistence, and effectiveness of the candidate biocontrol agents. This knowledge will help in small field tests, and in environmental safety evaluation. A good example is the recent discovery that at least some species of the mosquito pathogenic *Coelomomyces* (fungi) must go

[20] Such a scheme has been approved in principle by the 21st World Health Organization's Expert Committee on Insecticides.

through an alternate infective cycle in a cyclopid crustacean. Laboratory studies to maintain such cultures had often failed previously because pure water lacking microfauna was used as a medium.

No biocontrol agent can be of any value if it cannot be produced with relative ease. At the start of the investigation, it must therefore be determined whether or not mass propagation will be possible. If it is, the efforts necessary to obtain adequate amounts of the agent must be evaluated.

At the start of developmental studies (of microbial pathogens in particular), there exists a unique possibility to test human beings who will be exposed in the laboratory. All persons associated with the project should be in good health. Serum collected before they begin working with the agent should be stored frozen, to be checked later when serological tests are available. This should not be interpreted to mean that the exposed workers must not be protected. In fact, laboratory and research personnel should be instructed to handle the prospective agent with the utmost care — all laboratory accidents such as breakage and spillage must be reported and investigated. For all practical purposes, laboratory experimentation should be conducted as though one were dealing with a known infectious agent.

Stage II

For a pathogen showing a potential for becoming a useful control agent, comprehensive safety tests should be started during this stage. Short-term, and subacute, studies with laboratory animals should be conducted. The type of study and the possible effects to be looked for will vary greatly with the type of pathogen and its basic properties, as determined in Stage I (e.g. a pathogen whose growth characteristics indicate that it cannot grow under the conditions existing in the gastrointestinal tract — temperature, acidity, O_2 concentration, etc. would still be tested by the oral route, but with minimum short-term exposure). Emphasis must be placed on growth potential under more favourable conditions, which may exist, for example, on the skin, in the eyes, and possibly in the lungs. As another example, testing in tissue culture is a specialized and sensitive approach for viruses. However, it is of little value for other pathogens.

Although effects on various nontarget species will be studied in detail in Stage IV using controlled laboratory studies, and again in Stage V concurrent with operational uses, it is important that the effects on a selected number of nontarget species should have been studied in Stage II. Most laboratory animals used to assess human safety will be mammals. Acute and/or subacute tests will call for the use of invertebrates (bees, a crustacean, and a mollusc), aquatic vertebrates (1 or 2 species), and one or two birds. When selecting these subjects, it must be borne in mind that the fauna of the eventual use-locations may not lend themselves for laboratory rearing. Therefore, sensible substitutions (with species easily reared and controlled under experimental conditions) will have to be made.

As soon as some of the safety tests are completed, the results should be reviewed by a group of experts from national regulatory agencies and/or experts from UN Specialized Agencies such as WHO. Based on this review, the preliminary field trials of Stage III should be started. It is not necessary that long-term studies be completed before Stage III (which is still an experimental phase) begins. In America, for example, Stage III would fall under "experimental use permits" — these limit and control the extent, time-period and degree of use of control agents.

Stage III

The time limited field trials of this stage are designed primarily to assess the performance of the control agent, and to test optimal use application rates, formulations, timing, etc. These trials must be

conducted under strict supervision. Although the performance testing of the control agent is the primary aspect of Stage III, questions relating to safety must be considered at this phase as well. Such well-controlled investigations are especially suited to study environmental effects, including persistence under natural conditions, and adverse effects upon nontarget vertebrates and invertebrates. Pathogen levels before and immediately after treatment, and at various time periods thereafter, must be determined.

Long-term safety tests are usually rather expensive. Therefore, those assessing novel biocontrol agents would do well not to proceed with sophisticated research programs until preliminary field trials have shown tangible success and promise. However, once the decision has been taken to develop a selected entomopathogen to the level of a practical biocontrol agent, long-term studies will be required. From the beginning, these should include safety-related investigations in order to assure synchronized completion of the whole development program.

Stage IV

This stage can be viewed as transitional between Stages III and V. Some tests and observations concerning nontarget species will have been conducted in the laboratory in Stage II and in the field in Stage III. Experiments on nontarget vertebrates, especially on species that will be exposed at Stage V (i.e. during large-scale field use), should be continued under controlled laboratory conditions. Depending on the proposed use, representative fish, reptiles, birds, mammals, and nontarget invertebrates (especially insects and crustaceans) should be tested. The type of tests and the criteria for determining effects (if any) should be similar to the laboratory tests conducted under Stage II.

Second-year limited field trials would also fall under Stage IV. These field trials should try to correct failures that may have been encountered in earlier trials. They should also pursue favourable trends recorded with respect to formulations, and application technologies and frequencies. After completing Stage IV, the parameters of the control program should be defined, and data for a comprehensive judgement on the safety aspects must be available.

It should be emphasized that new personnel involved in the expanded field and laboratory testing should be monitored on a continuous basis for possible adverse health effects (see Stage I).

After completion of Stages I–IV, all data relevant to the control agent should again be reviewed by specialists. They must reach agreement as to whether or not to proceed to Stage V — large-scale, controlled trials. Should the safety data not support this step, the deficiencies should be identified and recommendations made on how to rectify the situation.

Stage V

At this point, large-scale operational field applications are undertaken to gather data on the ultimate effectiveness of the control agent. Long-range safety considerations, however, must also be incorporated into these trials. Several critical parameters must be established to assess any adverse effect on the environment and on public health. Suggestions and discussions concerning these parameters are discussed later. Such safety requirements imply that large-scale field applications have to be supervised (or at least monitored) by experts, for several years. This will give continued assurance that the control agent is continuing to fulfill all safety requirements. In fact, in certain areas, it may be advisable to conduct a review program every 3–5 years, to avoid problems that have occurred with chemical pesticides (e.g. harmful environmental effects and pest resistance).

It must be noted that in the United States, registration of a control agent will not be possible before satisfactory operational uses are reported and reviewed. The

suggested review between Stages IV and V would, in essence, still be a review resulting in extended experimental permits and temporary food tolerances (where applicable). Final registration would only be possible, after reporting and reviewing results of large-scale operational use.

Identification and Characterization of Biological Control Agents

Bacteria and Fungi

Criteria for identification are already established for pathogens belonging to these groups. Typing procedures as described in Bergey's Manual (Buchanan and Gibbons 1974) must be used. The identification of fungi is generally more difficult, and appropriate mycological expertise will be required. National reference centres (or organizations like the American Type Culture Collection) will be valuable resources for identification.

Laboratories and research groups dealing with new pathogens must be responsible for maintaining standard cultures for later comparison and deposition in reference laboratories. Standard cultures must be periodically checked for viability, to ensure that no potentially useful entomopathogens will ever be lost from them. Responsibly regulated exchange of cultures between scientists is highly desirable.

Concurrent with the identification process, growth characteristics (such as substrate, temperature, and humidity requirements of the pathogen) must be studied — this furnishes information on the sensitivity spectrum of the organism to antibiotics. A preliminary evaluation of the pathogen's genetic stability should be made. This is especially important in the case of fungi. Maintenance and use of standard seed cultures will lower the chances for inadvertent type changes.

Bacteria and especially fungi may produce very harmful toxins. The presence or absence of such toxins must be investigated by bioassays as well as by chemical methods. Assay methods must be adapted from those cases where mycotoxins and bacterial toxins have been demonstrated.

Protozoa

The identification of protozoa is even more difficult than typing of bacteria or fungi. Most criteria are based on morphology, and it has been shown that these may not be stable markers, particularly when external pressures such as substrate changes are applied. For the identification and characterization of entomopathogenic protozoa, much basic research is needed before standardized methods are available.

Several protozoa, which are human pathogens, such as plasmodia, trypanosomes, and leishmanias, have been studied extensively: identification and typing techniques used for them could be adapted for insect pathogens. It appears that careful serotyping will be the most promising approach to the detailed identification of insect-pathogenic protozoa.

Viruses

It has been repeatedly claimed that virus identification and characterization pose the most difficult problem of all. Insect viruses fall into several classes, based on their morphological appearance. A unique group are the baculoviruses (nuclear polyhedrosis viruses (NPVs), and granulosis viruses (GVs) — these do not seem to have counterparts among vertebrate or plant viruses. It has therefore been suggested that the baculoviruses show the best promise for pest control agents (WHO 1973; Summers et al. 1975). This does not mean that other viruses such as insect pox and cytoplasmic polyhedrosis viruses (CPVs) should not be considered. However, when characterizing them, the most searching attention must continue to be paid to their possible relationship to vertebrate viruses.

(1) Standardized methods to purify vi-

ruses and viral subunits such as virions and inclusion body proteins, must be used. The basic structural units of viruses can also be further broken down and analyzed. This will identify and characterize the proteins, glycoproteins, phosphoproteins, and enzymes embodied in the structure.

(2) Serological identification must be based upon the structural units — above all, the virions. Double gel-diffusion has been the usual technique employed for this. Serological identification must be extended beyond gel-diffusion to include virus neutralization, completent fixation, immuno-fluorescence, radio-immune reactions, etc.

(3) Based on the precise identification, reference or prototype viruses must be kept. Initially this can be done in individual laboratories. Later, viruses and standard reagents (sera) for identification must be deposited in national or international reference centres.

(4) The increased use and sophistication of tissue culture techniques will certainly facilitate virus identification. Plaquing techniques are beginning to emerge, and they can be expected to bring sophistication to virus characterization through cloning, plaque inhibition, and assessment of mutant strains.

Human Safety

Infections

Entomopathogens must be tested to determine whether or not they can cause overt infection of vertebrates, including man. The primary routes of exposure — oral, pulmonary, and dermal — must be considered. This is especially important for saprophytic organisms. The term "human pathogen" has lost much of its meaning since it became apparent that many normally nonpathogenic organisms (for example *Pseudomonas*) can cause problems under certain conditions. Full consideration must be given to the protection and health of not only healthy and well-fed humans, but also nutritionally deprived immunodepressed individuals, or those on antibiotic drugs.

With virus infections especially, there is a possibility of a persistent or inapparent infection. From several examples in mammalian virology we know that low level infection with or without shedding of virus can occur. Permanent or temporary incorporation of viral genomes into cell genomes are possible, with effects ranging from minimal (such as partial virus protein synthesis) to maximal expression (such as the formation of cancerous cells).

Noting that there is no evidence whatsoever that these observations are applicable to the baculoviruses of insects, it is nevertheless evident that our knowledge about the mechanism(s) leading to the subinfectious states referred to is far from complete. In fact, no standard technique can be suggested to detect inapparent involvement of viruses at the cellular level. However, the search for changed surface and internal antigens, and nucleic acid hybridization may be applicable to the demonstration of nonproductive viral infections.

Immune Reactions

Since the pathogen incorporates foreign proteins there is a possibility of immunological reactions involving the skin and the pneumo-bronchial system. Various degrees of immunological manifestations (allergies, sensitization, and anaphylaxis), must thus be searched for. When safety-testing insect pathogens, these possibilities must be considered in designing the necessary laboratory animal studies. Exposed humans must also be periodically checked for the development of allergies or sensitization.

No candidate biocontrol agent capable of providing signs of infection (overt or covert) in man and vertebrates, should ever be used for practical pest or vector control. However, certain forms of immunological reactions — although cause for extreme care — need not necessarily eliminate the

agent from further consideration, because the high-risk exposure group can be protected from excessive contact. Human and wildlife populations can be protected by keeping the general exposure low.

Toxins

The presence of toxins in microbial control agents is probably limited to bacteria and fungi (and possibly, some protozoa). Often, indeed, the toxin will be the insecticidal principle. The nature and/or presence of toxins should be determined along with the identification. Evaluation of the safety of toxins would follow that of chemical pesticides, calling for the determination of: the chemical nature of the toxin; the expected residue levels; and the toxicity for vertebrates. A comprehensive review of these factors would in most cases allow a judgement concerning the control agent's safety. This is especially applicable to situations where the toxin *per se* will be used as the control agent. Where the toxin will be produced as a by-product due to microbial growth, the situation is more complex. A determination of inadvertent toxin residues should then be made. Based on this, it should be possible to determine whether or not the exposure can be supported based on toxicological evaluations.

Risks to Humans

High Risk Group

The high risk group of the human population consists of persons working with the insect control agent in the laboratory or field. This small segment of the population must be adequately protected by aseptic techniques, respirators, and protective clothing. They should be both fully informed about the kind of control agent they are working with, and properly trained to avoid undue exposure. This particular population is, incidentally, the ideal one for monitoring on a short- and long-term basis.

General Population

The general population should never be exposed to a control agent before it has been exhaustively tested in laboratory animals. Effects are hardest to detect and evaluate in this group, because of the uncertainty about who was exposed to what and when. Nevertheless, survey programs for them will be prerequisite for the recording of epidemiological and public health record data. In principle, studies of the general population would only be indicated should there be evidence of existing danger in the high-risk population.

Prediction and Prevention of Possible Hazards

Decisions on whether or not hazards to humans exist, must be based on carefully controlled studies with laboratory animals. For each group of microbial control agents, different tests may be necessary. Basically, it must be shown that the agent is not infectious and/or toxic to the test species.

There is a special problem with safety tests for microbial control agents. In the case of chemical agents, a toxic level and a dose response can usually be established. Based on the no-effect level, a judgement and valid prediction of safe levels for human exposure can be made. With insect pathogens, this approach is not valid. To establish safety, the laboratory tests must be negative — especially with regard to infectivity. The interpretation of such negative tests is very difficult. The important question to ask is: Did the investigator use sufficiently sensitive and adequate methods for the detection of an effect had it occurred? This means that the mode of action and methods of detection in the permissive system (target species) must be known in order to determine that there was no effect in the nonpermissive system (nontarget species). In the performance of laboratory tests, basic principles of medical microbiology must be considered.

Techniques for detecting actions of pathogens should be adopted from the fields of infectious diseases in man and in animals.

A sizeable percentage of human beings are on a regimen of antibiotics or immunodepressive drugs or are otherwise in a state of lessened resistance to infectious diseases either as a result of nutritional deficiencies or diseases affecting the immune system. The competency of the immune system or antibiotic treatment can affect the outcome of a microbial infection. To determine if such impaired or altered states of health will affect the infection success of entomopathogens, animals whose immune responses have been suppressed or which are already on antibiotic treatment should be exposed (this applies especially to fungi).

Most laboratory tests will be performed using live animals. For certain pathogens, especially obligate intracellular parasites, the use of tissue culture may complement the studies on whole animals. Tissue culture has the advantage that direct cellular interactions can be studied, with proper control of external parameters such as temperature and nutritional state of the cells. On the other hand, tissue cultures lack the defence mechanisms (antibodies, lymphocytes, gastric digestion, etc.) of the living animals. In evaluating the results of tissue culture studies, these factors must be taken into account.

For each class of insect pathogens, we must develop guidelines and protocols to advise the investigator on what to look for, and how to detect adverse effects. Agencies in several countries are presently studying the design of relevant guidelines and protocols.

Together with the predictive studies discussed above, monitoring studies of the high risk exposure group must be undertaken. Two questions (what to look for, and how to look for it) are not easily answered for human exposure studies. Some techniques for isolation and identification of the pathogens, such as serological tests, may be adaptable from the animal studies. Probably the best approach to human health monitoring would be a complete and comprehensive periodic health check, including the testing of central nervous system responses, the cardiovascular system, and allergic reactions. Because of the usually small numbers of individuals involved, statistical analysis and comparison with the general population will be difficult. On the other hand, obvious changes — such as allergies and other immune reactions — are unlikely to escape detection.

The monitoring of the general population after extensive applications have been made (see Stage V above) is still more difficult than monitoring personnel employed in production and application. However, public health officials in treatment areas should be alerted to be on the watch for any unusual effects. Furthermore, allergic and other reactions encountered among exposed laboratory personnel should be kept in mind when observing the general public. The advantages of such an approach will be that the number of monitored individuals will be greater, and statistical evaluation will be possible through comparison with matched control groups.

A major factor affecting the hazard to the general population is the level of exposure. For this reason, it is important to study environmental levels of entomopathogens, and their persistence under natural and use conditions. These determinations of exposure levels in the environment, including insect control directed to food crops, will depend on the development of standardized bioassays. Because of the variable nature of such bioassays, the studies cannot be compared with residue analysis for chemical pesticides, and cannot be used for strict enforcement purposes. They should, nevertheless, be perfected, in the interest of obtaining a general picture of pathogen levels, and thus, of exposure levels.

As a last preventive measure, where applicable, information on antidotes to the pathogen must be available (see identification of pathogens). Should, despite all the

testing and monitoring recommended above, a human health hazard develop, it would be of the utmost importance that an antibiotic agent be available to protect the public. This situation, however, must remain a hypothetical one; because safety testing must be comprehensive, and evaluation of data conservative, before any entomopathogen is released in great quantity.

Environmental Safety

Many of the environmental safety aspects to be considered have already been treated in the preceding section. This is especially so in the descriptions of the proposed scheme for evaluation and safety-testing. Environmental safety is considered in those sections dealing with nontarget species. The basic concern is that introduction or augmentation of naturally occurring populations of predators, parasites, or pathogens of arthropods might prove deleterious to components of the biota other than the target pest and/or vector species.

Invertebrate Populations

In Stage II it is recommended that presumptive safety tests be conducted against certain standard organisms at appropriate life stages: where the appropriate stage is unknown, several larval forms and the adult stage should be checked. For example, for entomopathogens to be used against aquatic insect pests or vectors, dragonfly larvae, crustacea (crayfish and copepods), and molluscs would suffice. In Stage IV the testing should be based on representatives of the known invertebrate fauna of the areas to be treated. If, for example, commercial or edible species (e.g. shrimp, crabs, or oysters in receptor estuaries) occur in the test area these should be studied. Terrestrial systems are more difficult, but in Stage II, honeybees or other pollinators, silkworms, terrestrial nematodes, and soil Coleoptera might be used. In Stage IV the major nontarget species would have to be determined according to the proposed site of application, depending on the target habitat. Whenever possible, caged specimens should be employed, being positioned in appropriate sites for maximal exposure in the test area. As a general rule it would be wisest to commence testing with two principles in mind: (1) initial testing should be concentrated on four or five organisms not closely related to the target species; and (2) should these tests prove negative, subsequent ones should be extended to several taxa, closely related (perhaps at the generic level) to the target species. In all cases, organisms of economic importance to man or those that are major components of the ecosystem concerned, should be emphasized.

Vertebrate Populations

The testing of nontarget species of vertebrates in the laboratory (Stage II) should provide considerable insight into the selection of species to be tested in any given habitat in Stage IV. This will largely depend on the nature of the pathogen. Vertebrate pathogens (viral, bacterial, fungal, protozoal) and metazoan parasites sometimes show great specificity at the species or generic levels. In other cases, though, they may be almost ubiquitous. Considering the currently demonstrated health and environmental safety of entomopathogens (parasites and parasitoids of insects are even less hazardous) a selected species of fish (and its eggs, if relevant), an amphibian, a reptile, a bird, and a mammal, from the ecosystem in question, should suffice as subjects for overt infection tests before proceeding to a Stage V field trial.

Had these, or closely related forms, been tested in Stage II or IV, this stage could be eliminated. Aquatic systems (fish and amphibian) should be exposed to the pathogens by inoculation of the water and by feeding insects killed by the pathogen. Terrestrial forms (birds and mammals)

should be tested by intraperitoneal injection and by feeding them diseased insects. Reptiles, according to habitat, should be tested by either method. Wherever possible, caged specimens should be placed in appropriate sites for maximal exposure in the test area.

It should be mandatory during Stage V tests, that the same vertebrate species be sampled in longitudinal series from the test area and examined for evidence of overt infections. Should infections be demonstrated, this should be followed by serological tests. Sera samples should be retained from early tests for subsequent testing at 3–5-year intervals.

Plants

Certain candidate biocontrol agents, for example fungi, might damage seeds or other plant parts. At Stage II, a culture of filamentous algae should be used for test purposes. At Stage IV, if a fungus has to be tested against rice-field mosquitoes, it should be tested against planting-stage seedlings. It is not believed that other groups of insect parasites or pathogens could lead to major concern regarding plants. However, considering the ease of testing, an algal culture and a seedling plant should nevertheless be exposed to each agent in question.

Environmental Equilibrium

All biological systems tend to develop toward a state of equilibrium. A major criticism of chemical insecticides is that the addition of such extraneous factors has upset certain equilibria by inadvertently affecting their component parts. Yet, the purpose of pest and vector control always is to produce an environmental change. To produce maximum yields of food, it is necessary to halt natural depredation of herbivores or of plant diseases. Human maladies such as malaria, onchocerciasis, and yellow fever, are naturally occurring diseases whose transmission we wish to interrupt. To do so, *a priori*, involves an environmental change, for which a value judgement must be made.

The objective in testing human and environmental safety of candidate biocontrol agents should be to ensure that the change required is as small as possible, and directed only to the components of a given ecosystem (or rather, to as few of them as possible) to achieve the desired purpose. The same can be said for the use of chemical insecticides. In all cases, it is necessary to balance the acceptable levels of change against the benefit to be derived (e.g. improved public health, greater food production).

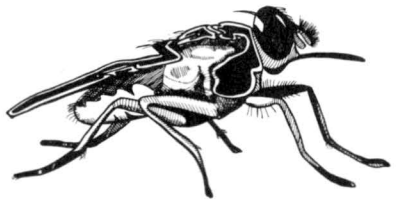
Recommended procedures for testing the safe use of various classes of biocontrol agents, especially through Stages I–IV, have been outlined. A further dimension remains to be considered for Stage V trials — environmental equilibrium. This is something that cannot be done in the laboratory and which requires an exhaustive knowledge of the components of the ecosystems being studied. A chemical or biological control method can kill fish or birds by acute toxicity (or infection), or by destroying the food chain necessary for their maintenance. The former can be studied directly, the latter only indirectly. For the proper conduct of Stage V, we must be able to monitor, throughout the trials, the population levels of different components of the ecosystem (producers, consumers, and degraders). Selected representatives of each category must be kept under close observation, to determine if, and when, a population imbalance occurs.

Such an indirect measure will provide broader information on host-specificity than would normally be available from the proposed laboratory tests. Additionally, it is fundamental to study the residual nature of the agent applied, and its activity and persistence throughout the ecosystem. Should the residual effect be too time-consuming, target species with short generation-times might develop resistance (as they have to certain chemical insecticides); if it is too brief, efficacy will suffer.

To evaluate such a situation, type cultures must be maintained. Ideally, the required studies should determine relative frequency of species. In practice, less quantitative information on seasonally adjusted relative abundance of ecosystem components selected by their trophic roles, before and after treatment, would suffice. It would also be pertinent to adapt Metcalf's recent Model Ecosystem approach, in connection with studies on biodegradibility of chemical insecticides, to determine the fate of biocontrol agents (especially aquatic ones) in a closed ecosystem.

Present concern about the human environmental safety aspects of chemical control methods has prompted a search for alternative methods of insect pest and vector control. Notable among these alternative methods is biocontrol, especially the use of predators, parasites, and pathogens of target species. As there is not yet a clear system for evaluating the efficacy and safety of proposed biocontrol agents, a workable framework for such a system has been offered. It is submitted that such a scheme would minimize development costs by outlining a logical series of stages in their development, and would prompt collaboration between the scientists representing the required skills (e.g. medical and agricultural entomologists, microbiologists, pathologists, toxicologists, ecologists, and industrial specialists). It would also furnish national and international agencies, concerned with testing and regulation, with a scale for determining the development status of any particular agent. In preparing these guidelines, it was recognized that whereas safety testing must be rigorous, excessive demands could not but have a detrimental effect on the development of practical biocontrol procedures. Finally, it is submitted that national and international agencies should collaborate in facilitating such efficacy and safety tests through an established network of collaborating centres at their respective levels.

The scheme proposed is based on these premises: (1) that identification and characterization of any candidate microbial control agent is of critical importance; and that this should be followed by laboratory tests for efficacy against target vectors, and ease of production; (2) presumptive safety tests for man and other nontarget species are then required; (3) strictly regulated field trials should follow; (4) advanced tests for human safety and host-specificity among nontarget species are now mandatory; and (5) supervised full-scale field tests should await the completion of stages I–IV. — R. Engler and A.A. Arata.



Training in Biological and Integrated Control

Biological control is the use of living organisms to control pests. The approach can be integrated with the selective use of chemical pesticides in ways designed to avoid harming naturally occurring agents. The target of a biological or an integrated control treatment usually is a single pest or vector species. However, any one environment usually is the home of a variety of kinds of pests: weeds, vertebrates, plant diseases, nematodes, as well as insects and mites. Therefore, it may be the site of a number of control treatments each applied more or less independently of the others, and each with pest destruction as its primary purpose.

One consequence of this is that preventive measures may be neglected. Another is that the different controls may not supplement one another. Indeed, they may counteract one another to some extent; some of the energy used in applying them may thus be wasted. A third consequence, and the most important one, is that chemical pesticides are added to the environment in quantities that can be superfluous, harmful, and inefficient.

There is growing need to reduce the amounts of chemical pesticides used to the minimum required to complete any job of pest control effectively. This means in part reducing the actual amounts applied, and in part, replacing some chemical control treatments with nonchemical control methods.

The reasons why the use of chemical pesticides should and will be reduced are various. Nonselective commercial pesticides can be inefficient — though they may alleviate the symptoms of pest attack quickly and effectively, they do not cure the ailments. Instead, and if not applied carefully in integrated programs, they can tend to intensify existing pest problems and to create new ones by killing biocontrol agents. They, or their degradation products or contaminants, can accumulate in the environment, harming crops, livestock, wildlife, and man. Moreover, they may not remain effective as pesticides, because pests can develop resistance and immunity to them. Environmental protection and public health legislation increasingly restrict their availability and limit their use. Also (and most importantly in practice) they are becoming so expensive as to be increasingly beyond the means of an increasing number of users — particularly those in developing countries.

The best available solution to all these problems is now generally recognized as pest management: the management of environments in ways designed to result in the best combination of minimum pest damage with minimum harm to useful elements that is commensurate with the information and resources available at the time. It is the third profession that deals with the harm caused by living organisms to people and their property, the others being the medical and veterinary professions. Like those older professions, it will develop a large corps of private practitioners (see Beirne 1967 and Peterson 1974). The potential manpower requirements for pest management in agriculture in the United States have been estimated by the U.S. Department of Agriculture at over 11 000 professional crop protection specialists and 168 000 assistants.

Pest management is the ecological approach to the prevention of pest damage, as distinct from the purely pesticidal approach. An environment-based integrated philosophy, it replaces pest-based approaches that are segregated from one another even if each is integrated within itself. It is the integration with one another of all the control programs used in that environment. It is a method that must continually improve in effectiveness as knowledge increases.

The desirable basic sequence of events in applying a pest management program is to: use physical procedures to deprive the pests of requirements for survival and increase; cause the pests to be killed by self-perpetuating, noncontaminating means; use other kinds of noncontaminating, selective pest-killing means; and, finally, use means that can have harmful side-effects or residual effects, but only when there is no remaining alternative. Or: (1) use cultural control methods; (2) alter permanently the ecology of the environment to the detriment of the pest species by (*a*) physical means, or (*b*) establishing there new biological control agents (including pest-resistant varieties of crops, as well as parasites and predators); (3) apply selective pesticides, especially biological ones such as microbial pathogens, if they are available; and (4), if or when those methods are not adequately effective, apply chemical pesticides at only the times and only in the minimum quantities needed to produce good effects and not undesirable ones, those times normally being determined by monitoring population changes of the pests and of their important natural enemies.

In a given environment, any one of these (or a combination of any two or more) may prove to be the best feasible solution. To generalize very broadly, the best answer in annual crop situations often may be a combination of (1) and (4); in forests and against vectors of diseases of man and livestock it may be (2); and in buildings and other closed environments it is often likely to be (4).

It is evident that a trained pest manager must be informed on all kinds of pests and of all possible control or management methods, and have practical experience with both of these as they relate to at least one main class of environments, (e.g. agricultural), and desirably to the others as well.

Systems for training people for research, advisory, or applied work in pest control have been largely fragmented, and indeed usually still are. For instance, training may be related to insects but not to weeds, to plant pathogens but not to harmful vertebrates, to pests of agriculture but not of structures, or to forestry pests but not to pests of veterinary or direct human significance. Often it may be related more to measures that kill pests rather than to ecological procedures for managing pest populations to keep them at low levels. The comprehensive, integrated approach (pest management) is one that requires a broad, comprehensive, and integrated training; both as an end in itself, and as a basis for specialization in some aspect of pests (or of their management, or of a related basic discipline).

Pest management training programs have been or are being developed in various universities in advanced countries. Tending to have certain characteristics in common, they usually cover all kinds of pests, pest environments, and management (including control) measures. They include a period of practical experience in the field, and are not based primarily on research theses. They more resemble in their general approach a medical or a veterinary training program than a typical MSc or PhD one.

They may differ from one another in subject emphasis, often because of their origins. Thus, a curriculum that developed from a university department of entomology may tend to emphasize insect pest management, to synonymize this term with integrated control, and perhaps to pay relatively little attention to weeds and to pest vertebrates. A curriculum that developed in a faculty of agriculture may

tend to emphasize agricultural pest management and to neglect that of forest pests or of disease vectors, and thus tend to synonymize pest management with agricultural pest management.

The organization of a pest management curriculum may be illustrated by that at Simon Fraser University (SFU) at Burnaby, British Columbia, Canada, leading to the degree of Master of Pest Management (MPM). It is proposed as the example primarily because it is the program with which the authors are most familiar. As it was developed in a new university it has been able to avoid influences of the history of the parent organization. It primarily attempts to produce generalists in pest management, though with some opportunity for specialization.

The SFU program consists essentially of four overlapping stages, as follows.

(1) A prerequisite education that includes undergraduate courses in plant and animal ecology, entomology and plant pathology, organic chemistry and biochemistry; but that otherwise may be predominantly in any of a number of areas that are relevant to pest management, from such central fields as biology, medicine, or agriculture to such peripheral ones as, for instance, economics, chemistry, engineering, or political science.

(2) A selection of graduate (i.e. postgraduate) courses on principles of pest management, which fall into three main groups: (*a*) on pests: insects and mites, nematodes and plant diseases, weeds, harmful vertebrates, etc., and how they may be managed; (*b*) on management procedures: cultural, physical, behavioural, biological, and chemical procedures, and their characteristics, mechanisms, strengths, weaknesses, interactions, and integration; and (*c*) on related subjects or processes of which some are subjects of separate courses, whereas others are incorporated in the above courses or, especially, in those referred to below (3). They include, notably: population dynamics, epidemiology, toxicology, parasitology, pathology, and physiology; characteristics of research, application, information and regulatory agencies; soils, agricultural and forest engineering, plant growth, and the like, as they relate to pests or to their management; relevant legislation and communication procedures of various kinds; and sociology, economics, biometrics, and computer science.

(3) Broad professional, or practical, experience in the field. This is provided in five courses given largely by more than 60 experts (listed by Beirne 1974) from outside the University who are specialists in aspects of practical pest management. The courses cover practical applications of the process mentioned under (2*a*) to pests of different kinds of environments: agricultural, orchard, forest, savannah, urban, and industrial.

(1) An individual study in depth by each student, on a subject tailored to each particular case and normally concerning the aspect of pest management in which the student hopes to be employed.

The practical parts of the curricula (3, above) of some other universities (cf. Obein and Motooka 1973) appear to be more specialized in that the student may gain his practical experience through a period of employment at a research station that is related to a particular class of environment or of type of crop. Both kinds of programs, the more general and the more specialized, are useful. The more general is the most applicable to training for the management of vector populations, for it involves management of a wide variety of habitats: forest, swamp, grassland, savannah, urban, and agricultural.

In advanced countries, pest managers are employed in research, development, consulting, advisory, regulatory, inspection, monitoring, or educational capacities, and in the application of pest management procedures. They are employed by various levels of government and by industry or are in private practice. Their activities relate to agriculture, forestry, public health, housing, recreation, environmental protection, and/or food storage and processing. Similar job opportunities should appear in

due course in developing countries as the same needs become recognized there.

In developing countries, though, the initial emphasis must be on agricultural pest management. In most of these countries agriculture is still the mainstay of the economy, and (as was highlighted at the Food Conference organized by the United Nations in Rome in 1974) food production is often inadequate. It is therefore important that the first trained pest managers should be deployed in agriculture and related fields.[21]

Another aspect of particular importance in developing countries is the control of arthropods that are vectors of diseases of man and livestock. Tsetse flies are only one example. Those diseases, in addition to their direct significance to human health, contribute seriously to the food production problem both by causing loss of man-hours and by preventing large tracts from being adequately inhabited and farmed to the extent that their agricultural potentials warrant. There is therefore an important role for pest managers in dealing with such diseases in both rural and urban situations. The latter pose demands for public health officers dealing with pest and vector problems of cities and towns, and with issues concerning the food-production and tourist industries. As developing countries progress, additional needs for pest managers, similar to those in advanced countries, will appear.

Though the principles and procedures of pest management are applicable in all parts of the world, a training program given in one region is not necessarily the optimum for people who will work in other regions. Its practical segment having to be based on pest situations of the local region, it cannot deal adequately (if at all) with situations characteristic of other parts of the world. For instance, the practical courses of the SFU curriculum cover pests of coniferous forests but not of tropical agriculture; whereas, those of a pest management curriculum at a university in the tropics would probably do the opposite. Therefore, there is clearly a need for programs to be established in, and for, the tropical and subtropical regions.

The SFU program, aimed at producing general practitioners, is an example of one kind of pest management curriculum. Other curricula may emphasize a particular aspect (as with those that concentrate on agricultural pest management) and be more extensive than the MPM program — as is the Doctor in Pest Management program advocated by Peterson (1973) for the University of Hawaii. At the other extreme, it may merely be an undergraduate major that includes courses in pestology and pest management or a diploma program consisting largely of practical training and thus aimed at giving the pest management approach to people already working in pest control, who may or may not have academic training, or who require refresher courses. Which of these is developed at any institution will depend on the needs existing in the region, and the resources that are available to meet them.

An adequate program must, however, be at least at the Master's level. It must be comprehensive and train generalists. Most pest managers will be employed as specialists but, as with any profession, a specialist should first be trained as a generalist if his decisions and judgements are to be soundly based. A specialist in only one aspect of pest management cannot properly be entitled a professional pest manager.

Three main kinds of training programs appear to be needed in developing countries, as elsewhere. Because they overlap, all three might be organized by the one institution in any one region.

(1) Short courses of a week or so, each covering an individual pest problem and

[21] While recognizing the general validity of this statement, it should be remembered that there are exceptional vector-borne disease situations (e.g. onchocerciasis in the Volta River basin) where the initial priority must be in the field of public health entomology and not economic entomology, for the reasons mentioned in the following paragraph (ed).

its management (e.g. tsetse flies); or individual procedures and their capabilities (e.g. pest or pesticide monitoring). These courses should be aimed primarily at people already working in these subjects, or who plan to do so but lack formal training. The courses should be given in the field by people with practical experience with the subjects of the courses. Each course should be both given in different regions, and updated for regular repetition in any one region. Ultimately they would evolve into what would be largely refresher courses.

(2) Structured programs of several months duration perhaps leading to a diploma in Pest Management. Such a program might be one of several kinds. It might be on pest management as a whole, especially its applied aspects, perhaps somewhat resembling the practical courses of SFU's MPM program; being offered to people already working in pest control, and who may or may not have previous relevant academic training. Or, it might deal in detail with a particular class of pest problem; for instance, arthropod vectors of diseases of man and livestock. Again, its subject might be a particular management procedure, for instance biological and cultural controls, such a program being offered to people already trained and experienced in pest management or related subjects. These programs may ultimately evolve into refresher programs.

(3) Full professional programs in pest management, producing general practitioners in the subject, having a Master's degree. As in the SFU program (see also Beirne 1974) and elsewhere (see Obein and Motooka 1973; Peterson 1973), any such program need not or should not require a major research thesis. It should cover both the principle and practice of pest management, and give the student some direct experience with work on practical problems. Such a program would have to be given by the university level. Desirably, it should involve experts in practical pest management from agencies outside the university, as does SFU's program. Consequently, a university that has research or scientific service agencies nearby, concerned with some aspects of pest damage prevention and control (such as a government research laboratory or a station of the Commonwealth Institute of Biological Control) offers especially suitable circumstances for a Master's program in pest management.

However, there is an alternative: that a graduate (postgraduate) program should incorporate a research thesis, so that those concerned become familiar with the research process and are thus capable of initiating and directing the research activities needed to obtain the information on which practical pest management programs can be based. Moreover, the research theses themselves would contribute to that information.

The number of critically important pest problems and regional differences in languages and economics demonstrate that needs exist for a number of courses and programs of the kinds indicated above in the tropical and subtropical regions of the world. They should be given as far as feasible by indigenous personnel, who should take the initiative in planning, organizing, and developing them. The locations for training programs will in fact depend primarily on locations of universities that are both willing and (perhaps with aid from advanced countries initially) able to develop and operate them.

One such centre should emphasize pest management of staple food crops such as millet, sorghum, and maize. Another might emphasize pest prevention in stored food products. One must deal with the management of arthropod vectors of diseases of man and livestock. Another perhaps could specialize in management of aquatic weeds and animals. Others might concentrate on management of pests of specific crops such as cocoa, coffee, coconuts, and sugarcane, that are of critical economic importance to particular countries. Some must be related to particular language situations in Africa: francophone, anglophone, or others. All must take the broad, integrated, management approach to dealing with pest problems,

the people teaching them having the breadth of outlook and training needed to teach one integrated program rather than to teach a number of separate subjects individually.

Because the ultimate consequences of the programs will be beneficial to humanity, advanced countries have a responsibility to assist in their organization and development. This responsibility might take the form of offering expert advice on the nature, organization, feasibility, and suitability of planned programs; of training pest-management scientists from developing countries who will return to staff the local training programs, as well as to participate in pest and vector control operations; and of assisting with material facilities. But the responsibility for operating the programs, as well as for the initiative in organizing and developing them, should rest with the developing countries — it must not have to depend on continuing assistance from advanced ones.

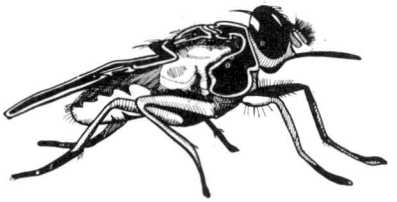
Pest management training tailored to the needs of students from the third world could enable developing countries to learn from past mistakes in advanced ones where highly detrimental side effects sometimes followed short-sighted control procedures. Pest control in developing countries has involved makeshift, holding-the-line, actions. One reason has been that the knowledge of the pests necessary for development of long-term management programs simply never accumulated. Another reason had its roots in the "non-permanency" of former colonial research workers — they understandably did not have sufficient time to obtain comprehensive pictures of pest ecosystems before developing control measures.

Though indigenous personnel are being trained in or for developing countries, their numbers are still far too small to obtain the data from diverse fields that are needed for development of effective management programs. Moreover, this shortage of trained manpower has too often led to promising researchers being channelled into administration. Furthermore, pressures from governments for quick and visible results has affected detrimentally the establishment and persistence of long-term organized research programs. Tsetse control having been a major victim of this short-sighted approach, it is thus a subject where a broader pest management outlook could be especially productive.

Pest management needs specialists as well as many general practitioners. Therefore, in addition to new pest management curricula, conventional, specialized graduate programs based on research theses should be continued in developing countries. Whenever possible, though, the theses' subjects should focus on fields basic to the concept of pest management: ecology, population dynamics, toxicology, epidemiology, pathology, and the like. Desirably, of course, a pest manager should be both a generalist and a specialist: trained first as a general practitioner in the subject, and thereafter educated as a specialist on some aspect of the subject.

Good communications must be developed, if the best use is to be made of any pest management training programs that may be established in and for developing countries. There are indications (e.g. IDRC 1974) that there is room for improvement in existing communication systems. Administrators must be informed about the results and the value of the pest management approach so that they may assist students to secure training, often perhaps in another country; whereas, potential students must be fully informed on the existence and the nature of all relevant educational facilities available to them (wherever those facilities are located).

Pest management is based on the management of people to facilitate their manipulation of pest populations to mankind's advantage. Suitable education and adequate communications are, therefore, among the most important procedures for the management of pest and vector problems. — B.P. Beirne and S.N. Okiwelu.



Biocontrol Prospects in Future Integrated Tsetse Control Programs

Previous chapters make it plain that so far, the biological control of tsetse flies has been virtually synonymous with short-lived mass production and field release of *Syntomosphyrum* parasitoids. In this chapter, the term biocontrol is being used in its strictest sense to convey the deliberate use of predators, parasitoids, parasites, and pathogens against undesirable insects. Autocidal or genetic control is regarded as an altogether separate element for possible inclusion in future integrated control methodologies. Neither will we discuss such draconian measures as area-wide bush clearance and game destruction and highly selective culling practices, such as the elimination of bushbuck and duiker (good hosts for *Trypanosoma rhodesiense*) from the immediate vicinity of human settlements (van den Berghe and Lambrecht 1963). Equally unconsidered is ecological displacement, the process whereby the perceptive David Livingstone (1874) noted that houseflies (*Musca domestica*) when freshly introduced from Europe into New Zealand, displaced the indigenous bluebottle flies (Calliphorinae) — he added the rueful comment, ". . .but what European insect will follow us and extirpate the tsetse?. . .Here there is so much room for everything."

It is simply accepted that the growing trend towards increasingly selective control will continue. Future integrated control programs, based on carefully planned land use projections (Vos 1975) and forming part of coordinated activities towards socioeconomic betterment, will certainly place a continuing demand on the special qualities (including persistence) of various chemical pesticides. These, though, will be employed in more and more selective ways, a sensible approach that will see their intermeshing with other types of measures combining effectiveness against *Glossina* with the least possible hazard to nontarget organisms. Integrated methodologies will have a place for already available "novel" procedures such as autocidal control, and yet-to-be-developed ones like disturbance of the normal female tsetse reproductive cycle so as to cause the larva to be aborted (Tobe 1974), something that might be managed through the use of insect growth regulators (Denlinge 1975). Limited brush control by employing selective herbicides (combining habitat denial to tsetse with the possibility of controlled pasture improvements for cattle raising) will accompany any or all of these techniques; among which biological control as defined above is likely to be prominent.

As is evident from the voluminous literature cited, a great deal that bears directly and indirectly upon this topic has been published. Moreover, much of what has been written has been reviewed again and again. A good deal of it concerns facultative predators undoubtedly contributing to natural population-limiting factors (see Predators), but scarcely likely to form the basis of practical biocontrol procedures. From Swynnerton (1936) through Buxton (1955) to Nash (1969) there is general agreement that parasitoids and perhaps parasites and pathogens have considerable potential in mankind's fight against the transmission of trypanosomiases to humans and domestic animals by species of *Glossina*. There is less of a consensus, though, on precisely how such a potential is to be realized.

One reason for this is that earlier at-

tempts to compile the scattered information have been far from complete (not that full completion is claimed in the present case). Thus Austen (1903) and Jenkins (1964) both overlooked Livingstone's (1857, p. 499) record of what was probably a cicindelid beetle preying upon tsetse and other flies in southern Africa. Jenkins also missed Hindle's (1914) speculation as to the likelihood of tiger beetles (Cicindelidae) attacking tsetse, because of the coincidence of these two insects' streamside habitats. Austen admittedly quoted de Castelnau (1858, p. 986) for an intriguing piece of information. This concerned an indigenous belief that tsetse give birth to winged miniatures of themselves, illustrated for the edification of a visiting European by South African Bushmen who opened the abdomen of what was represented as a pregnant female, out of which emerged three little flies ready for flight. Interestingly enough, Austen (1903) failed to appreciate that this may have constituted the first published record of a parasitoid of *Glossina*.

The record is of particular significance in that as recently as 1955, Buxton mentioned that we knew of no parasitoids from adult tsetse as distinct from puparia. The occurrence of such "lost" records in the relatively small late-nineteenth century travel literature pertaining to Africa, suggests that an exhaustive search of the voluminous biological and medical literature concerning this continent over the past century or so might well prove even more rewarding. This is underlined by the circumstance that, after writing the last sentence and suddenly recollecting Wenyon's (1926) useful list of Invertebrate Hosts of Trypanosomidae (Vol. II, p. 1404–1414) the volume was taken from the shelf — resulting in the turning up of a record that has eluded most recent reviewers, that of Lloyd (1924) for a herpetomonad flagellate (genus *Leptomonas*) from *G. morsitans* in Nigeria (presumably the record mentioned without a literature citation by Buxton 1955, p. 404).

Because of the obvious adverse effects of parasitoids upon *Glossina* populations, and the dramatic impact upon the observer of the total removal of tsetse adults from the scene by successful predator strikes, these two types of biocontrol agents have received most attention to date. The information on predators is, however, virtually all diurnal. Buxton (1955) suggested that there might be important nocturnal predators on tsetse, ranging from bark-searching bats to reduviid bugs and other predatory Hemiptera. Improving technology, notably the recent availability of electro-optic night viewing devices operating on a light-amplification principle, opens new routes to the field investigation of this question without disturbing either resting tsetse flies or those of their natural enemies active after dark.

However, it is exceptional for insect predators to be restricted to one particular type of prey. Thus the *Pheidole* ants that under favourable circumstances may destroy concentrations of tsetse puparia (p. 29 and Ford 1940), *Paltothyreus tarsatus* (see Carpenter 1912) and other species that have on occasion been observed to carry away larval *Glossina* during their very brief period of vulnerability at the surface of the larviposition site (p. 47), and the asilid flies and hersiliid spiders discussed by Gruvel (p. 47), are all facultative predators. Indeed, as Southon (1959) showed for *Hersilia setifrons*, the coincidence of 1400 spiders and 75 tsetse adults per hectare involves a weekly predation rate of only 17%. Moreover, each spider feeds but once weekly, and *Glossina* constitutes a mere 1% of the hersiliids' prey. Under such circumstances, and recognizing how little we yet know about the overall ecology of any one species of predator, it seems rather unlikely that ant, asilid, spider, and other such populations can be manipulated (without recourse to major environmental management such as widespread burning after several years' exclusion of fire, as discussed by Ford 1940) to bring about any material reduction of the numbers of *Glossina*.

What *is* of immediate relevance to inte-

grated control in this context, is that lacking detailed prior knowledge of tsetse/natural enemy interrelationships, it is only too tempting to use chemical pesticides and herbicides unwisely. In this connection, even certain plants may play a part, however small, in the natural limitation of tsetse populations. Thus Dawe (1922) and Najera (1932) referred to the entrapping of tsetse flies by leaves of the grass *Melinis minutiflora* Beauv. (the generic name is misspelt *Melinus* in the citation of Najera's paper by Jenkins 1964). Dalziel (1937) indicated that this indigenous tropical African member of the Gramineae was "probably carried to Brazil by the slave trade" and that it had entered into cultivation in America for fodder and pasture purposes. He stated that the grass has both "a strong odour perceptible at a distance" and a viscid exudate in which insects can become entangled. However, while noting that it is also alleged to have deterrent effects on mosquito breeding (unsubstantiated by experiments at Kumasi, Ghana) and repellency to reptiles and carnivorous animals, Dalziel expressed doubt as to the anti-tsetse qualities of *M. minutiflora*.

Especially in view of the known choice of certain types of vegetation as resting sites by adult tsetse flies, and waiving for the present the obviously complex basis of such choice, there is nevertheless room for more specific studies of the interrelationships between plants and tsetse, as the heavy-handed bush clearances of the past (Swynnerton 1936; van den Berghe and Lambrecht 1963) are replaced by increasingly selective integrated control approaches. Notable among these is the highly selective use of knapsack sprayers to apply DDT wettable powder to individual resting sites for 100-day effectiveness (Na'isa 1971) without posing any material environmental hazard (Laird 1973) other than, perhaps, harm to such facultative predators as *Hersilia* spiders.

Of course, we enter deeply into the realm of subjectivity as soon as we attempt to estimate the "seriousness" of environmental hazards. On the one hand, we must keep human values in mind. The realities of this horn of the dilemma could hardly be better put than by Kangwagye (personal communication): "However, the present problem is that the tsetse and trypanosomiasis work require immediate results particularly when you are fighting a sleeping sickness epidemic or when land is required for immediate livestock development." On the other hand, we are working in an era of high awareness of the ease with which nontarget organisms can be harmed by heavy-handed control methods. Even so, one of the most telling arguments against the gloom-and-doom school of environmentalist extremism relates to South Africa's eradication of tseste flies from Zululand (Toit 1947; Toit et al. 1954; also see p. 30) by the massive aerial application of DDT (assisted in some areas by the use of ground-based DDT smoke generators, and by discriminative clearing). Today, after more than 20 years, Zululand has become an important cattle-producing area the inhabitants of which are afforded incomparably better opportunities than they enjoyed previously, in an environment still boasting an incredibly rich diversity of wildlife.

Dr Kangwagye's remark quoted in the previous paragraph was made in the context of one of the research needs highlighted in the Report of the Scientific Advisory Group convened at the Memorial University of Newfoundland, St. John's, Canada, 25–29 March, 1974 (IDRC 1974). This recommendation urged the fostering and support of research within Africa toward the characterization of candidate biocontrol agents "with further attention to their natural habitat, host preferences, behaviour, development, distribution, and the construction of relevant life-tables. . ." in the interests of the establishment of priorities "for trial inoculative introductions of candidate biological control agents from one region of Africa to another, and/or their propagation in very large amounts for inundative field releases."

The need for characterization of such agents quite eloquently expresses one of

our major impediments to implementing biocontrol against tsetse flies at this time. We know a little about quite a number of the more obvious potential biological control agents, but still have a distressingly limited knowledge of the bionomics of any one of them. This applies particularly to the microbial agents (Summers et al. 1975; Laird 1975b). Earlier chapters in this book make the point perfectly clear, leading to the inevitable conclusion that of all such agents yet known, the parasitoids have been most thoroughly investigated.

Can we, then, at this stage, seriously contemplate the mass production and controlled field release of parasitoids as a material factor in tsetse control? It has, after all, been done before. In point of fact, the first such trial took place a little over half a century ago, when Lamborn bred large numbers of the eulophid microhymenopteran now known as *Syntomosphyrum albiclavus* (using *Sarcophaga* flies as the laboratory host, long before *Glossina* was first colonized), and released some 277 000 of them in a peninsula of Lake Nyasa, now Lake Malawi in the modern republic of Malawi. Briefly, as described on p. 62, the natural incidence of *Syntomosphyrum* infection (of *G. m. morsitans* puparia), rose from 0.2 to 6.8%, then returned to the original level. Soon afterwards there was an inconclusive attempt along similar lines in northern Nigeria, and in 1931 Nash (1933a), working in modern Tanzania, bred *Syntomosphyrum* sp. in *Chrysomyia* puparia to such good effect that 3.5 million were released against *G. m. centralis*. Eventually, by early 1932, 13.75 million parasitoids had been liberated. Once again, there was only a short-lived indication of positive results. One further such effort in the 1930s, and the post-mortems, are summarized on p. 61.

Latterly, the facts that *Syntomosphyrum* (30–40 per puparium) is usually present in less than 1% of *Glossina* puparia, whereas the much larger mutillid wasps or "velvet ants" (one per puparium) commonly parasitize 20% of them, have focused attention on the latter group. This is the more so in that although from 3500 to 4000 species of mutillids (mostly within the single genus *Mutilla*) had been recognized nearly 50 years ago (Mickel 1928), those shown to be parasitoids of tsetse still remain altogether unknown in the most populous zone of the entire range of tsetse flies — West Africa. Other mutillids of unknown host associations are known from this region, from which *Mutilla senegalensis* had been described (in 1831) several years before it had even been suggested that these hymenopterans parasitize other insects (Mickel 1928), while *Dolichomutilla guineensis* var. *voltensis* was described from a single female from the Upper Volta River by Bradley and Bequaert (1923). The latter authors also reported taxonomically but not biologically, upon known species of *Odontomutilla* and *Smicromyrme* from Cameroon.

Nevertheless, from the vast West African focus of human trypanosomiasis and nagana disease, no mutillids have ever been recorded from tsetse despite the intensive studies of *Glossina* spp. that have been undertaken there. The best-known of the species from southern African tsetse, *Mutilla glossinae*, readily parasitizes puparia of *G. morsitans* and less readily, those of *Sarcophaga* sp. (Lamborn 1925). As only small numbers have been produced in the laboratory by comparison with the very much smaller *Syntomosphyrum*, which attacks a range of dipterous hosts other than tsetse, there have not been any field trials of mutillids to date (p. 65). Perhaps, the rapidly increasing potential for mass-producing *Glossina* spp. justifies a second look at this situation?

As with biological control of other insect pests and vectors, two general approaches present themselves. The first and most satisfactory (of which there are successful examples from economic entomology, particularly where introduced pests have been fought by subsequent deliberate introductions of their natural enemies from the original habitat) involves confrontation of the pest population by a biocontrol agent that will become permanently established

and by its action reduce the pest population to an economically acceptable level. The second is to make massive releases of suitable natural enemies at such a time that they can suppress the pest population effectively, even if only for a comparatively short period. The obvious disadvantages of the latter method are that breeding of large numbers of the natural enemy is obligatory, as are periodic releases, entailing recurrent expenditure. The early trials with *Syntomosphyrum* showed that after releases ceased, the level of parasitism soon fell to the "natural" level.

Bearing in mind the vast areas occupied by ecologically different species of *Glossina,* and their seasonal variation in numbers and distribution, it is virtually impossible that the introduction of any single parasitoid species into an area will result in widespread successful control of tsetse, or even that mass-releases of a single species of parasitoid in adequate numbers would be successful over the whole ecological range of *Glossina* spp. What should be aimed at is adequate biological control of each dangerous species of *Glossina* in the ecological situations most important to man. This may well entail the application of both methods, using different species of parasitoids in different areas.

The choice of *Syntomosphyrum* for the trials earlier referred to, was made solely because it was easily bred in large numbers — according to Saunders (1964b) "any Cyclorrhaphous puparia of suitable size" will do. This is hardly a sound criterion for selecting a species for use in a biological control trial. With hindsight, it was obviously premature to conduct such field experiments in the absence of far more basic information than was available at the time. It took the trials themselves to raise the suspicion that tsetse flies are not the prime natural host of *Syntomosphyrum* spp. A parasitoid should be selected because of its suitability in several basic characteristics, notably the obvious one that *Glossina* spp. should constitute its preferred targets. Other such points to be considered are its ecological adaptability to the environment it will encounter, high searching ability enabling it to find host puparia that may be scattered over a wide area, facility to increase rapidly when hosts are present as well as to maintain its population when hosts are scarce, and capacity to survive periods of adverse weather, etc. It may well be impossible to find a parasitoid with all these advantageous characteristics. Obviously, though, that species exhibiting most of them is best suited for any trial, and hence selection for laboratory colonization. Its suitability on any of the counts listed can only be determined by extensive experiments. This in itself necessitates availability of numbers of the parasitoid, and hence some degree of mass production.

Two groups certainly warranting immediate attention are the mutillids and bombyliids. For this reason they were dealt with in some detail in a previous chapter. Both contain species that in different areas sometimes cause high degrees of parasitism of *Glossina* puparia. With particular respect to the mutillids, which have never been recorded as *Glossina* parasitoids in West Africa, there are possibilities of introducing various species into areas from which they are as yet absent. Before attempting such introductions, it will be necessary to study the biology, host-finding capabilities and preferences of each such species and their ecological tolerances (and especially as regards bombyliids, it may well be difficult to develop laboratory breeding techniques). It will also be necessary to ensure that the laboratory population bred for field-release is well-suited to field survival and establishment. Perhaps, genetic heterogeneity is a more desirable character than homogeneity in this respect (Beard 1964).

To have a scientifically acceptable baseline against which the results of any such region-to-region introductions can be accurately monitored, it will also be essential to undertake purposeful prior surveys, for example mutillids in West African tsetse, to confirm the present belief that *Glossina* is in fact free from these parasitoids there.

In this connection, it is useful to refer to Table 50 in Buxton (1955), which summarized much of the then-available information on parasitoids of tsetse. Under the three species of *Mutilla* mentioned therein, there are no listings outside of southern or southeastern Africa; but there are no country-columns for any part of francophone West Africa anyway. Also, the only parasitoid noted from Zululand is *Syntomosphyrum glossinae*; which Harris (1930), (cited by Buxton), had long before reported from South African *G. pallidipes* — along with *Mutilla auxiliaris, Thyridanthrax abruptus,* and *Trichopria capensis robustior.* Furthermore, a year prior to the publication of Buxton's book, the systematic puparial survey conducted in connection with South Africa's post-World War II tsetse eradication campaign had led to: the confirmation of Harris' records; their extension to *G. brevipalpis* (and in the case of *Mutilla auxiliaris,* to *G. austeni* as well); the discovery of a fourth, *Thyridanthrax brevifacies,* in all three hosts; and the realization that a new bombyliid parasitoid was "very common in the tsetse region" (Fiedler and Kluge 1954).

Therefore, care must be exercised in assembling the available information and even more so in purposefully searching for new data, before we make generalizations. This is especially important in a field such as this where our present understanding of the natural occurrence and incidence of potential biocontrol agents so largely reflects the distribution of, and intensity of the work undertaken by, a relatively small handful of individuals particularly interested in this subject.

None of the other *Glossina* parasitoids appear to have real promise as biocontrol agents on present evidence, tsetse flies seeming in general to be accidental hosts for them. However, the fact that in some areas, which have been subjected to intensive study, a whole complex of tsetse parasitoids collectively impose a measurable degree of natural control over *Glossina* populations, indicates that we would do well to bear the implications of this in mind when planning any large-scale operations with chemical pesticides anywhere in the flybelt. For example, Fiedler (*in* Nash 1969, p. 200), "during the elimination of the Zululand *pallidipes* belt. . . revealed that aerial applications of insecticide eliminated the parasites before the tsetse." This lent urgency to the achievement of full success in the undertaking, in which connection, after having produced evidence suggesting that parasitoids exert "a noticeable controlling influence on the larger types of tsetse flies only" (and have a virtually negligible effect on the smaller species such as *G. austeni, G. swynnertoni,* and *G. p. newsteadi*), Fiedler and Kluge (1954) were able to end their paper with the statement that thanks to the success of the DDT-based operation in Zululand, "the density of tsetses and parasites has dropped to such an extent as to render further biological experiments impossible."

As regards the use of nonpersistent pesticides, in integrated control programs, against *Glossina,* Nash (1969) asked whether if aerial application of such compounds "was confined in Rhodesia to the cold months, when Chorley found that *Thyridanthrax* and *mutillids* were quiescent, would the great hatch of the parasites in the ensuing hot months be capable of destroying any tsetse that had survived the spraying? We have no idea, because we do not even know whether these parasites can reach the more deeply buried tsetse pupae."

Clearly, then, there is much still to be learned regarding tsetse/parasitoid interrelationships, if only to open the way to sound pest management practices. As to the practical employment of mass-reared parasitoids against *Glossina,* it must be accepted that the general biology and habits of tsetse flies as a whole do not make them really good candidates for "classical" biocontrol by means of insect parasites and predators. Nevertheless, exploration of two relevant areas is certainly warranted: (1) the prospects for importing parasitoids from hosts other than *Glossina,* occurring

elsewhere; and (2) the feasibility of adding to the effectiveness of already-present natural control agents of the larger West African tsetse flies such as the nagana-transmitters of the *G. fusca* group, and perhaps of the medium-sized sleeping-sickness vectors like *G. morsitans* and *G. palpalis*, by importing mutillids and bombyliids (mass-reared in hosts other than tsetse) from other parts of Africa.

Following earlier recommendations along these lines, the emphasis placed upon such research needs in IDRC (1974) led in 1975 to a 2-year grant of $42 400 on Biological Control Tsetse (Kenya), "For the Commonwealth Institute of Biological Control, Curepe, Trinidad, to enable the Institute to contribute to the eradication of trypanosomiasis through research into the biological control of the tsetse fly" (IDRC 1975). This project, is initially expected to concentrate upon the biologies of East African tsetse parasitoids of the genera *Mutilla* and (*Thyridanthrax*) *Exhyalanthrax* in part (see p. 65, 66).

With his customary acuteness, although writing in a period when the whole subject of enemies and parasites of tsetse was receiving little attention, Buxton (1955, p. 399) answered those who "would say that it is useless to attempt to attack *Glossina* in its own home by enemies with which the insect has been in equilibrium for millenia." He submitted that: "This would be true enough if the African countryside was also static. But it is not: man is changing it all the time, and some of his changes may be favourable to certain of the enemies of *Glossina*." Buxton then, in the infancy of applied invertebrate pathology, went on to draw attention to Steinhaus' (1946, 1949) pioneer texts, and to emphasize the prevailing dominant role of the USA and Canada in this emerging discipline. Drawing attention to the particular difficulty of controlling forest insects by conventional means, he declared that: "At the present moment Canada, more than any other country, is disposed to undertake long-range research on infections (by bacteria, fungi, viruses, protozoa) in all types of forest insects. . ..Now tsetse-flies, ranging in relatively small numbers through great areas of woodlands, are also difficult to attack; they might indeed, even be regarded as forest insects. It might then, perhaps, be to our advantage to study them in the same way, and for the same reason."

Buxton then proceeded to give examples. Insofar as invertebrate pathogens and parasites are concerned, he emphasized Thomson's (1947) discovery of "*Mermis*" (one each in only three out of 1500 adult *G. morsitans*) as apparently causing "serious inconvenience to the fly"; Nash's (1933a) phycomycete fungus that, occurring "in as many as 20 per cent of wild flies at a certain season, . . .might have contributed to the great decrease in the numbers of flies which occurred after one particular period of very heavy rain and flood", and unspecified fungi responsible for "the death of whole boxes of flies" in the laboratory in modern Kinshasa, Zaïre (van Hoof and Henrard cited in Buxton 1955); and *"Bacterium mathisi"* (p. 78), pathogenic to *G. morsitans* (and several other muscoid flies, also even Orthoptera and Lepidoptera, but not mosquitoes). The latter organism was isolated from flies emerging in a Paris laboratory from puparia flown from what is now Tanzania (Roubaud and Treillard 1935, 1936). As indicated earlier in the book, this coccobacillus was easily cultured *in vitro*. It caused a fatal septicaemia in flies infected contaminatively by contact with the skin and hair of guinea pigs deliberately soiled with the culture, which was unfortunately lost. The organism has not been rediscovered in recent years, although a number of other records of bacteria from *Glossina* have accumulated.

Since the appearance of Buxton's book, perhaps the most promising pathogens and noninsectan parasites to have been described from tsetse flies are the strain of the fungus, *Absidia repens* found by Vey (1971) in *G. fusca congolensis* puparia in the Central African Republic (p. 80); the virus-like particles from the nuclei of mid-

gut epithelial cells of *G. f. fuscipes* reared at Basel from Uganda puparia and thought by Jenni and Steiger (1974) to be possibly related to a low emergence rate and high mortality of young flies (p. 82); and the undescribed mermithids from Upper Voltan *G. tachinoides* and Nigerian *G. palpalis, G. longipalpis,* and *G. m. morsitans* dealt with at some length earlier.

On existing information, we are thus faced with a quite remarkably small choice of potential microbial control agents from *Glossina*, by comparison with other major vectors of disease. Mosquitoes and blackflies, for example, harbour representatives of all the taxa just mentioned, and a wide range of others too. Of course, these Diptera with their aquatic larvae offer a wider range of possibilities for infection of the immature stages, as indeed do dung- and mud-breeding muscoid and tabanid flies more closely related to *Glossina*. In particular, *Musca domestica* and near relatives commonly harbour microsporidan protozoa. At first sight it seems strange that these should not have been detected in tsetse; unless indeed this was the true identity of "*Myxosporidium heibergi*," described from the gut of *G. palpalis* by Dutton et al. (1907), which proved to be the case for a parasite of bryozoans originally named *Myxosporidium bryozoides* Korotneff 1892, but later transferred to *Nosema* by Labbé (1899). A new genus of mermithid nematodes recently discovered in Mongolian *Musca autumnalis* and *Haematobia/Lyperosia*[22] provides timely evidence that a more intensive search for such candidate biocontrol agents in Muscidae might well widen the range of pathogens, parasites, and parasitoids meriting trial against tsetse.

At present, those microorganisms and parasites specific for mosquitoes and blackflies, and generally conceded a priority as really promising candidate microbial control agents, include mermithid nematodes, microsporidan protozoa, and (in the case of mosquitoes — one unconfirmed record only for blackflies) *Coelomomyces* fungi. As regards organisms of broader host range, strains of *Bacillus sphaericus* and the fungus *Metarrhizium anisopliae* are of much current interest as potential mosquito control agents too. In economic entomology, products based on *Bacillus thuringiensis* and its endotoxin have been widely marketed for years (largely for use against lepidopterous pests), and as mentioned in the Preface, the United States Environmental Protection Agency, on 1 December 1975, granted the first-ever label bearing their registration for a "viral insecticide" (an NPV that kills two major crop pests, the cotton bollworm and the tobacco budworm). Moreover, Henry (1975) is engaged in the large-scale field-testing of a microsporidan, *Nosema locustae*, against rangeland grasshoppers in Montana, and entomophthoraceous fungi are at an advanced stage of development for use against forest (Otvos 1976) and other insect pests.

Returning to vectors, a product based upon the mosquito mermithid *Romanomermis culicivorax* (equals in part *Reesimermis nielseni*) has now been registered in the USA, where it went onto the market in June 1976 under the name "Skeeter Doom" (Fairfax Biological Lab., Clinton Corners, New York). Also, recent information[23] indicates that during 1974/75 there was a successful introduction of a fungal pathogen of tabanids (until lately thought to be a *Coelomomyces*, and named *C. milkoi*, but currently being transferred to a new genus) into a natural horsefly population in Kazakhstan, USSR. Health and environmental safety relating to entomopathogenic viruses are summarized by WHO (1973), and Summers et al. (1975), whereas Ignoffo et al. (1973) fur-

[22] Personal communication from Prof I.A. Rubtsov to M. Laird, Leningrad, 3 May 1976.

[23] Personal communication from Mrs Rimma Andreeva and Dr A.M. Dubitskii to M. Laird, Kiev and Alma-Ata, April/May 1976.

nish convincing evidence of the noninfectivity of *Romanomermis culicivorax* for a wide range of nontarget organisms — the only insects other than mosquitoes that this mermithid has been shown to invade (so far, only in the laboratory) are blackfly larvae (Finney 1975; Hansen and Hansen 1976). No *Coelomomyces* fungi have yet been transferred to hosts other than the natural ones.

Microsporidans of insects had not been so transferred until very recently, when Undeen's (1975) success in bringing about infections of mammalian cells *in vitro* by *Nosema algerae* of mosquitoes was followed up by the demonstration of transitory, localized infections in white mice (Undeen and Alger 1976). It is of course a far cry from establishing an insect microsporidan in isolated cells of mammalian origin, to achieving enduring infections in the tissues of the living mammals. Ignoffo (p. 52–57 in Summers et al. 1975) has made this point with specific regard to entomopathogenic baculoviruses, stressing that a tissue quite insusceptible to a microorganism in the intact organism (furnished with infinitely complex defence mechanisms), may support that entity's multiplication when explanted into a culture medium. Nevertheless, the transitory infections described by Undeen and Alger (1976) emphasize the profound importance of adequate safety studies of all candidate microbial control agents, prior to field trials. Still more recently, this same intensively studied microsporidan has proved to infect backswimmers (*Notonecta undulata*, Hemiptera) to which diseased *Anopheles quadrimaculatus* larvae were fed. It would, therefore, be wise to conduct especially searching health and environmental safety tests upon any microsporidan candidate for microbial control — not that any such agent has yet been isolated from *Glossina* anyway (p. 184).

Much safety-related work has also been undertaken with respect to *Bacillus sphaericus* and *Metarrhizium anisopliae*, without disturbing data coming to light. These two organisms have the great advantage of being mass-producible by existing technology. Bearing in mind one of the recommendations in IDRC (1974) that "where invertebrate pathogens already in use and commercially available are known to affect Diptera, their effects on *Glossina* should be ascertained," they — and certainly *Bacillus thuringiensis* and its available metabolic products — are held to merit laboratory evaluation against tsetse flies in one or more of the institutions where self-maintaining colonies are now established. There would be obvious advantage in undertaking such investigations at African institutions, where, in the event of encouraging preliminary results, immediate projection into initial field trials would be feasible. Other parasites and pathogens from nontsetse natural hosts that might also be included in such evaluation programs, might include organisms from tabanids, such as "*Coelomomyces*" *milkoi* (p. 184) and another entity from the USSR, the mermithid *Eurymermis elongata* (Rubtzov et al. 1972), also the muscoid fly mermithids from Mongolia mentioned on p. 184.

The feasibility of mass producing biocontrol agents such as those cited above has already been discussed. It remains to be considered how they might best be shipped (Fisher 1964; Flanders and Bay 1964; Louw 1964 — re avoidance of mandatory disinsection) and applied in field trials, and eventually, practical field control programs. Some of the specific questions to be asked in these connections cannot possibly be answered until more is known about the bionomics of the agents in question. For example, we now understand the aquatic route followed by mermithids and their mode of entry to static-water mosquito larvae and turbulent-water blackfly larvae. How, though, do mermithids gain entry to *Glossina*?[24]

If such invasion normally takes place into immature tsetse, the act of applying mermithids to *Glossina* larviposition sites

[24] See footnote p. 87.

would necessarily be as careful and selective, to avoid wasting infective material, as is the selective chemical spraying of adult resting sites. That is to say, knapsack sprayers rather than aerial application equipment, and well-trained spraymen, would probably be essential. This might also be so for parasitoids, and for some microbial control agents gaining entry to the host in the larval or puparial stage. Parasitoids, however, might be more economically employed by infecting surplus puparia in tsetse mass-production centres, and hand-seeding these in likely larviposition sites rather than simply releasing the adult mutillids or bombyliids there.

Recollecting the claim of Roubaud and Treillard (1935) to have achieved contaminative infection of *G. morsitans* with "*Bacterium mathisi*," it seems worth considering the suitability of such an organism for two very different methods of application: (1) via aerial application in the manner of chemical pesticides and for that matter commercial preparations of *Bacillus thuringiensis*; and (2) through the use of bait stations. Of course, the presumptuousness of making such a forecast for "*B. mathisi*" is well-recognized, as the organism was lost to sight in the mid-1930s. (There was a recent claim that a strain had been rediscovered in Nigeria, but Briggs (personal communication 1975) ascertained during a visit to the institution concerned that this is not so.)

As for such a bacterium or some baculovirus lending itself to contaminative infection by way of the mouthparts of adult tsetse, the selectivity offered by the bait-station route has a certain appeal as regards answering the particular nonchemical need posed earlier; that is, for the control of *Glossina* in areas of human and domestic stock high population density, where the sterile male procedure is unattractive because these males do not have their own vectorial capacity impaired. The use of pheromones and other attractants in association with traps has already been advocated as a means of control in its own right (Vale and Hargrove 1975). Especially now that the latter investigators' intriguing results in augmenting female *G. pallidipes* catches four-fold by increasing the dosage of attractant ox-odour have moved the technique a little closer to practicality, and in view of recent advances with respect to pheromones of *Glossina* (Langley and Pimley 1976; Nash et al. 1976), the prospect of not destroying the tsetse coming to the traps but rather of letting them return into the "wild" population to spread contaminative infections still further, has definite appeal. Also, Pinhão and Grácio (1975) have claimed that *Glossina* commonly mates more than once under natural conditions, and indeed, that multiple mating is possibly the rule among at least young females. This, if confirmed, clearly reduces the feasibility of successful use of male sterility in tsetse control programs. On the other hand, it heightens the chances of spreading contaminative infections among "wild" flies, for example by means of insects that mate again after visiting an attractant-baited "trap" from which escape is assured via an exit channel where general body-surface contamination with the microbial agent is certain. As a theoretical model for such a trap, the elaborate pollination mechanisms of some insect-attracting orchids come immediately to mind.

In conclusion, it is evident that a measure of practical tsetse control by means of parasitoids is more likely in the foreseeable future than is such exploitation of nematode parasites and microbial pathogens. Nevertheless, the rapid move towards meaningful utilization of the two latter groups against other vectors, and the equally rapid advances in our knowledge of "ultimately relevant" aspects of tsetse biology, ecology, and control, offer some hope of an eventually valuable ingredient in integrated control programs. It is submitted that this fully justifies an immediate intensification of studies of (and training concerning!) this topic in nature and in the laboratory, directed towards the earliest possible field trials, so conducted as to assure accurate monitoring of their results. — M. Laird and F.J. Simmonds.

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(Abbreviations: CCTA — *Committee for Technical Cooperation in Africa South of the Sahara,* COPR — *Centre for Overseas Pest Research, EATRO — East African Trypanosomiasis Research Organisation,* FAO — *Food and Agriculture Organization of the United Nations,* IAEA — *International Atomic Energy Agency,* IEMVT — *Institut d'élevage et de médecine vétérinaire des pays tropicaux,* ISCTRC — *International Scientific Council for Trypanosomiasis Research and Control* (of OAU), NAS — *National Academy of Sciences,* (of USA), NITR — *Nigerian Institute for Trypanosomiasis Research,* OAU — *Organization for African Unity,* OCCGE — *Organisation de coordination et de coopération pour la lutte contre les grandes endémies,* ORSTOM — *Office de la recherche scientifique et technique Outre-Mer,* STRC — *Scientific, Technical and Research Commission* (of OAU), TPRI — *Tropical Pesticides Research Institute,* WHO — *World Health Organization.*)

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